VOLUME XXXI AUGUST, 1952 NUMBER 8

The Journal of Clinical Investigation

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

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The Journal of Clinical Investigation

Printed by the Lancaster Press, Inc., Prince and Lemon Sts., Lancaster, Pa. Published by the American Society for Clinical Investigation. Publication Office, c/o Presbyterian Hospital, 622 West 168th St., New York 32, N. Y.

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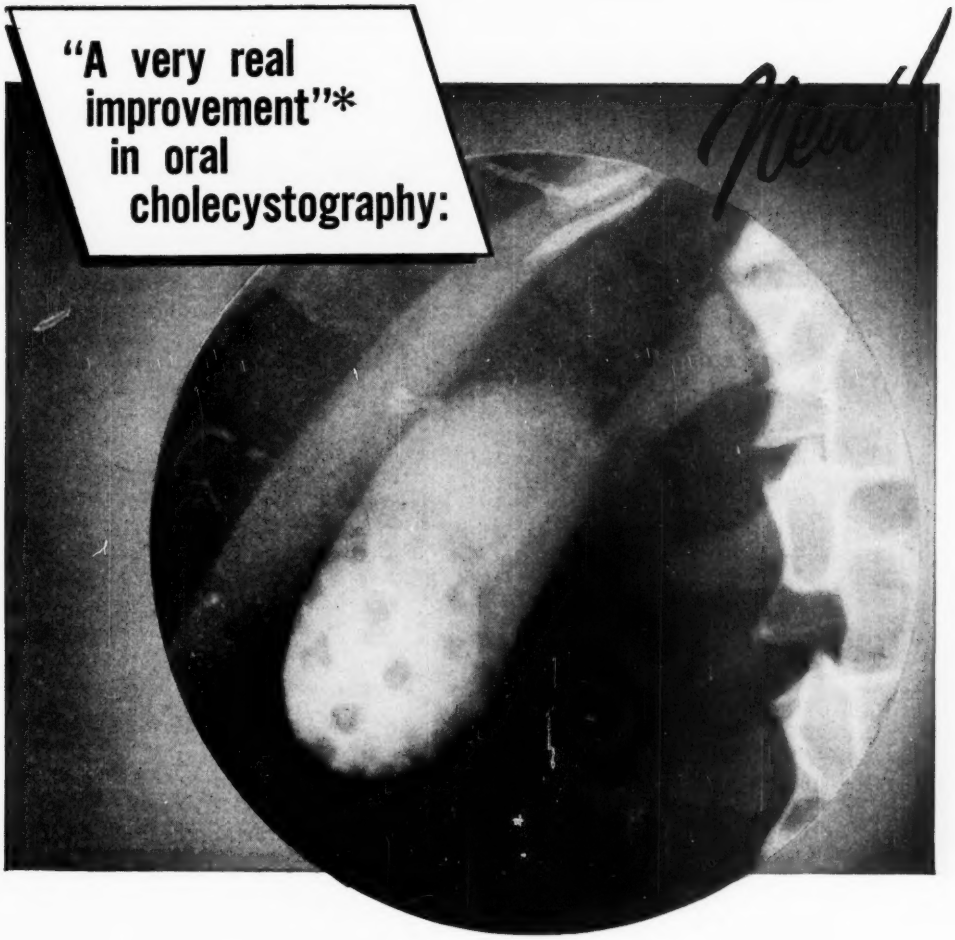

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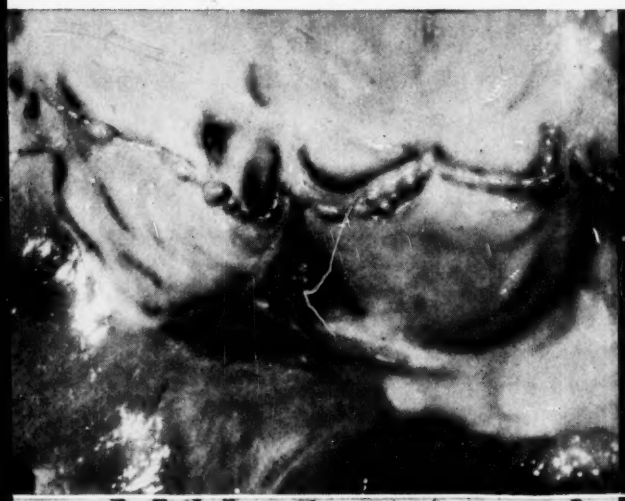
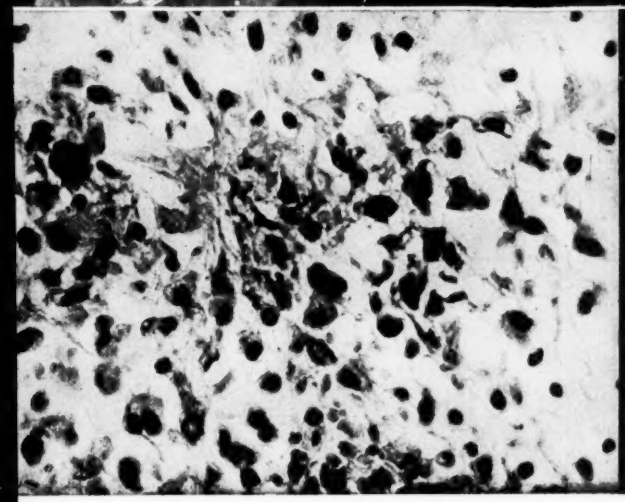
response in rheumatic fever



q. Does cortisone influence the heart lesions of rheumatic fever?

a. Early cortisone administration suppresses and in some cases may even prevent serious cardiac damage.



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BLOOD PYRUVIC ACID RESPONSE TO INTRAVENOUS GLUCOSE OR INSULIN IN THE NORMAL AND IN PATIENTS WITH LIVER DISEASE AND WITH DIABETES MELLITUS [1]

By DONALD S. AMATUZIO, NORMAN SHRIFTER, FRANCIS L. STUTZMAN, AND SAMUEL NESBITT

(From the Veterans Administration Hospital, and the Departments of Medicine and Surgery, University of Minnesota Medical School, Minneapolis, Minn.)

(Submitted for publication March 8, 1952; accepted June 3, 1952)

The conversion of glucose to pyruvic acid in normal metabolism has been intensively investigated. The present study was undertaken to ascertain blood pyruvic acid response to the intravenous administration of glucose or of insulin in normals, in patients with liver disease, and in patients with diabetes mellitus.

MATERIAL

Determination of pyruvic acid and of glucose in the blood was done by Friedemann's (1) modification of the Lu method (2) and the Benedict method (3), respectively.

The control group consisted of 28 healthy men ranging in age from 25 to 50 years. The series investigated by means of intravenously administered glucose included 15 patients with portal cirrhosis of the liver (three in hepatic coma), and 23 patients with controlled mild and severe diabetes mellitus (11 requiring 25 to 40 units of protamine zinc insulin daily). A total of 0.5 gm. of glucose per kilo of body weight was administered intravenously within 30 minutes, and blood was analyzed for pyruvic acid and glucose at 0, 30, 60, 120, and 180 minutes. The effect of 12 units of intravenously administered crystalline insulin was studied on 11 fasting normal subjects and five fasting diabetics requiring insulin.

RESULTS

In the control group the blood pyruvic acid response to glucose was similar to that described by others (4–6). At 30 and 60 minutes there was a statistically significant rise to 1.34 ± 0.23 mgm. % and 1.34 ± 0.28 mgm.%, respectively (Table I); the significance of difference between the means of correlated measures was $p < 0.01$. In 12 patients with compensated cirrhosis of the liver the blood pyruvic acid rise was similar to the control group (Table I); the significance of difference between the means of correlated measures at 30 and 60 minutes was $p < 0.01$. Figures 1 and 2 reveal the mean pyruvic acid and their maximum

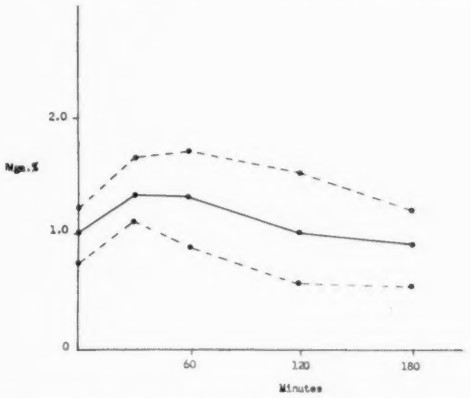

FIG. 1. THE RANGE AND MEAN PYRUVIC ACID RESPONSE TO INTRAVENOUS GLUCOSE IN 17 NORMAL MALES

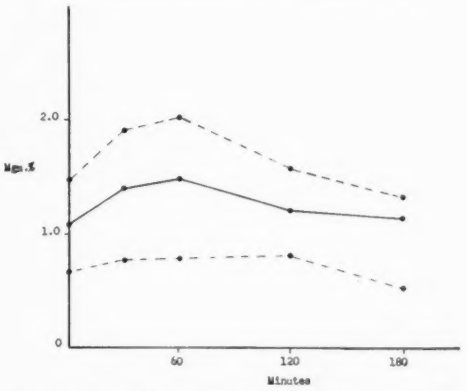

FIG. 2. THE RANGE AND MEAN PYRUVIC ACID FOUND AFTER INTRAVENOUS GLUCOSE IN 12 PATIENTS WITH CIRRHOSIS OF THE LIVER

[1] Published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

TABLE I

*Blood pyruvic acid response to intravenous glucose in normal, diabetes mellitus and cirrhosis of liver**

Time in minutes	Normal			Mild diabetes mellitus			Severe diabetes mellitus			Cirrhosis of liver		
	No.	Glucose	Pyruvic acid	No.	Glucose	Pyruvic acid	No.	Glucose	Pyruvic acid	No.	Glucose	Pyruvic acid
		mgm. %	*mgm. %*		*mgm. %*	*mgm. %*		*mgm. %*	*mgm. %*		*mgm. %*	*mgm. %*
0	17	82.5± 10.4	1.03± 0.17	12	110.3± 18.6	1.17± 0.23	11	222.6± 101.9	1.16± 0.33	12	82.6± 8.6	1.08± 0.39
30	17	220.7± 36.5	1.34± 0.23	12	282.9± 70.3	1.13± 0.26	11	362.5± 99.6	0.95± 0.29	12	221.0± 34.3	1.41± 0.43
60	17	133.7± 41.5	1.34± 0.36	12	205.1± 48.4	1.24± 0.26	11	346.6± 101.5	1.11± 0.22	12	142.0± 37.8	1.49± 0.45
120	17	81.4± 16.9	1.02± 0.39	12	142.3± 36.3	0.97± 0.26	11	285.1± 83.8	1.18± 0.30	12	80.7± 14.0	1.21± 0.63
180	17	75.5± 18.2	0.92± 0.26	12	112.7± 25.8	0.90± 0.28	11	256.6± 89.1	1.27± 0.39	12	82.5± 10.4	1.15± 0.80

* The mean and standard deviations.

and minimum values in the normal and in cirrhosis of the liver. Three patients were studied during hepatic coma. These patients had marked abnormalities of the bromsulfalein, fractional serum bilirubin, 24-hour urine urobilinogen, prothrombin time, total cholesterol and cholesterol esters, cephalin cholesterol flocculation, zinc sulfate turbidity, thymol turbidity, alkaline phosphatase, and plasma proteins including albumin-globulin ratio. Figure 3 illustrates the blood pyruvic acid rise following administration of glucose in these three patients. In each instance the rising blood pyruvic acid reveals the inability of the liver to assimilate pyruvic acid.

Twenty-three patients with diabetes mellitus (11 not requiring insulin) were similarly studied. The blood pyruvic acid values failed to respond to glucose as in the normal subjects. Instead, in mild diabetics the blood pyruvic acid showed no change at the 30-minute interval after intravenous glucose (Table I), and it was only suggestive of being statistically significant on comparing the normal to the mild diabetics ($t = 2.06$, $0.05 < p < 0.10$). Figure 4 graphically reveals in mild diabetes mellitus (the mean values with their maximum and minimum values) a delayed response of the blood pyruvic acid with the rise occurring at 60 minutes. In severe diabetes mellitus the blood pyruvic acid showed a drop at the 30-minute interval. A statistically significant difference was found at 30 minutes on comparing the normal to the severe diabetics ($t = 4.88$, $p < 0.01$). Figure 5 reveals the mean and the observed maximum and

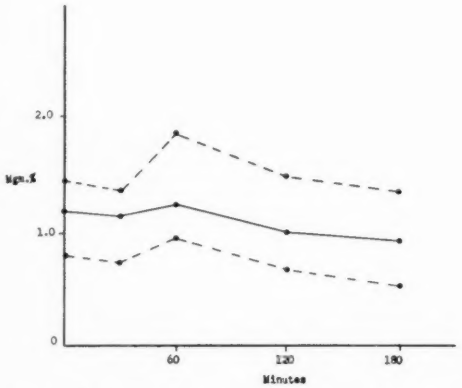

FIG. 4. THE MEAN AND RANGE OF PYRUVIC ACID IN 12 MILD DIABETICS AFTER INTRAVENOUS GLUCOSE

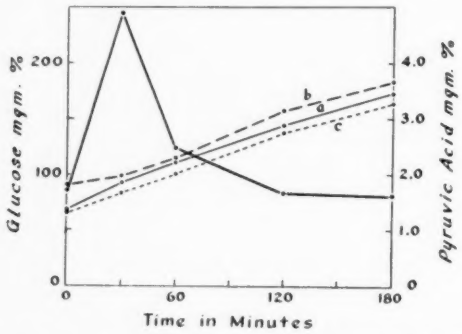

FIG. 3. THE BLOOD PYRUVIC ACID RESPONSE IN THREE PATIENTS (a, b, c) IN HEPATIC COMA TO I.V. GLUCOSE (MEAN VALUE)

minimum pyruvic acid values and there is noted a fall at the 30-minute interval with a prolonged rise to 120 minutes after intravenous glucose.

After the administration of 12 units of crystalline insulin intravenously in both the control group and in patients with diabetes mellitus, there was a rise in the blood pyruvic acid along with the drop

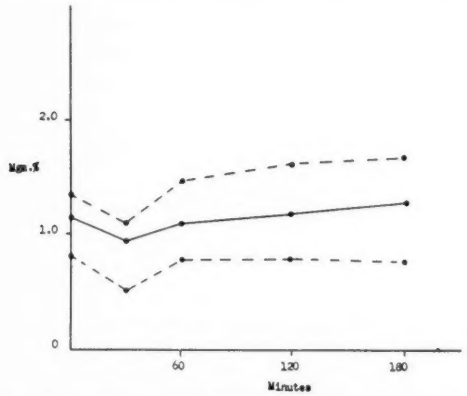

FIG. 5. THE MEAN AND RANGE OF PYRUVIC ACID IN 11 SEVERE DIABETICS AFTER INTRAVENOUS GLUCOSE

in blood glucose (Table II); the significance of difference between the means of correlated measures was $p < 0.01$ at 30 and 60 minutes. Figure 6 shows the pyruvic acid response in normal individuals to intravenous insulin.

TABLE II

*Blood pyruvic acid response to intravenous insulin in the normal and diabetes mellitus**

Time in minutes	Normal			Diabetes mellitus		
	No.	Glucose	Pyruvic acid	No.	Glucose	Pyruvic acid
		mgm. %	*mgm. %*		*mgm. %*	*mgm. %*
0	11	89.0 ± 10.0	1.02 ± 0.19	5	267.6 ± 40.3	1.04 ± 0.13
30	11	39.1 ± 14.6	1.35 ± 0.28	5	183.8 ± 28.0	1.74 ± 0.05
60	11	52.3 ± 11.3	1.60 ± 0.56	5	154.2 ± 26.1	1.45 ± 0.07
120	11	66.5 ± 18.5	1.23 ± 0.44	5	149.2 ± 33.4	1.12 ± 0.10
180	11	75.5 ± 11.7	1.12 ± 0.42	5	146.4 ± 33.9	1.18 ± 0.03

* The mean and standard deviations.

The analysis of variance of the fasting pyruvic acid in normal men and patients with compensated cirrhosis of the liver and diabetes mellitus (mild and severe) revealed the means to be not significantly different ($F = 1.27$).

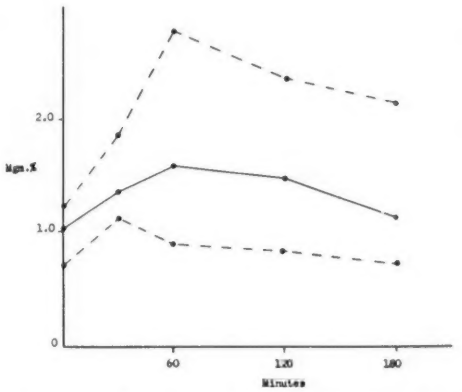

FIG. 6. THE RANGE AND MEAN PYRUVIC ACID IN 11 NORMAL MEN AFTER INTRAVENOUS INSULIN

DISCUSSION

The utilization of glucose in normal subjects is associated with the formation of intermediary products. A rise in blood pyruvic acid follows the administration of glucose (4–6), and this response may be associated with the action of insulin on the normal metabolism of glucose and pyruvic acid.

In patients with compensated cirrhosis of the liver, glucose utilization is apparently adequate. No diabetic glucose tolerance response was observed in the 15 patients studied. The ability of the liver to remove pyruvic acid also appears adequate in patients with compensated cirrhosis. In three consecutively studied patients in hepatic coma, however, an accumulation of the blood pyruvic acid occurred following the administration of glucose. The rising blood pyruvic acid is related to the inability of the liver to assimilate this metabolite (7) as in normal or compensated cirrhosis.

Reports in the literature with regard to the pyruvic acid response to glucose in diabetes mellitus are conflicting (5, 8–10). It is helpful to separate the mild from the severe diabetics. In diabetes mellitus the blood pyruvic acid failed to rise as in the normal men after intravenous glucose. In patients with mild diabetes mellitus not requiring insulin, a delayed rise in the pyruvic acid was found with the rise occurring at 60 minutes (Fig-

ure 4). This was only suggestive of being statistically significant at 30 minutes. However, in patients with severe diabetes mellitus requiring insulin, a statistically significant change of the pyruvic acid at 30 minutes occurred with a delayed rise to 120 minutes (Figure 5). The cause for the fall in pyruvic acid at 30 minutes is not known. The difference in the delayed response in the blood pyruvic acid as noted in mild and severe diabetes is apparently related to degree of insulin deficiency of the pancreas and is indirectly dependent upon the blood glucose level. It has been found that the rate of glucose utilization depends on the blood glucose levels in both normal and depancreatized dogs (11). If glucose was maintained at a high level in depancreatized dogs, the blood pyruvic acid rose (12). It is apparent that the pyruvic acid response after glucose is related to the degree of impaired glucose utilization in diabetes mellitus.

The insulin tolerance test in normal subjects and in patients with diabetes mellitus showed a rise in the blood pyruvic acid associated with the fall in the blood glucose level. This is in agreement with the observations of Gillman and Goldberg (6). It has been reported also that insulin produced no increase of blood pyruvic acid in the normal or in diabetic subjects (5, 13). However, this disagreement may be explained by factors regulating body needs. It is known that glucose may either be stored as glycogen or oxidized anaerobically to pyruvic acid and the conflicting findings are apparently related to body needs regarding either the storage of glucose or its oxidation to pyruvic acid.

SUMMARY

After the administration of glucose a statistically significant rise in the blood pyruvic acid occurred at 30 and 60 minutes in 17 normal men and 12 patients with compensated cirrhosis. An abnormal accumulation of blood pyruvate occurred with a normal glucose tolerance curve in three patients in liver coma. A suggestive significant delayed rise in the pyruvic acid was found after glucose administration in 12 patients with mild diabetes mellitus requiring no insulin. A further delayed response of the blood pyruvic acid was found in 11 severe cases of diabetes mellitus which was significant. The degree of delay is apparently related to the degree of insulin deficiency of the pancreas and indirectly dependent upon the blood glucose level.

A significant rise in the blood pyruvic acid was noted after the administration of crystalline insulin in 11 normal subjects. A similar response was noted in five patients with diabetes mellitus.

No significant difference was found on comparing the fasting mean blood pyruvic acid of the normal men, and patients with diabetes mellitus and compensated cirrhosis of the liver.

REFERENCES

1. Friedemann, T. E., and Haugen, G. E., Pyruvic acid. II. The determination of keto acids in blood and urine. J. Biol. Chem., 1943, **147**, 415.
2a. Lu, G. D., Studies on the metabolism of pyruvic acid in normal and vitamin B_1-deficient states. I. A rapid, specific and sensitive method for the estimation of blood pyruvate. Biochem. J., 1939, **33**, 249.
b. Lu, G. D., Studies on the metabolism of pyruvic acid in normal and vitamin B-deficient states. II. Blood pyruvate levels in the rat, pigeon, rabbit and man. III. The relation of blood pyruvate to cardiac changes. Biochem. J., 1939, **33**, 774.
3. Benedict, S. R., The determination of blood sugar. J. Biol. Chem., 1925, **64**, 207.
4. Bueding, E., Stein, M. H., and Wortis, H., The formation of pyruvic acid following glucose ingestion in man. J. Biol. Chem., 1941, **137**, 793.
5. Klein, D., The effects of administration of glucose and insulin on blood pyruvate and lactate in diabetes mellitus. J. Biol. Chem., 1942, **145**, 35.
6. Gillman, T., and Goldberg, L., Investigations on blood pyruvic acid. II. The effects of administering glucose, insulin and adrenaline on blood pyruvic acid in normal adults. South African J. M. Sc., 1943, **8**, 156.
7. Amatuzio, D. S., and Nesbitt, S., A study of pyruvic acid in the blood and spinal fluid of patients with liver disease with and without hepatic coma. J. Clin. Invest., 1950, **29**, 1486.
8. Bueding, E., Wortis, H., and Fein, H. D., Pyruvic acid metabolism in diabetes mellitus. Am. J. M. Sc., 1942, **204**, 838.
9. Himwich, W. A., and Himwich, H. E., Pyruvic acid in exercising depancreatized dogs and diabetic patients. J. Biol. Chem., 1946, **165**, 513.

10. Miller, M., Bueding, E., Strauch, R. O., Owens, J., and Woodward, H., Studies on pyruvate and citrate metabolism in man and animal. Proc. Am. Diabetes A., 1949, **9**, 83.

11. Hechter, O., Levine, R., and Soskin, S., Relationship between sugar concentration and glycogenetic action of insulin on rat diaphragm *in vitro*. Proc. Soc. Exper. Biol. & Med., 1941, **46**, 390.

12. Bueding, E., Fazenkas, J. F., Herrlich, H., and Himwich, H. E., Effect of insulin on pyruvic acid formation in depancreatized dogs. Science, 1942, **95**, 282.

13. Elliot, K. A. C., Rivers, T. D., Elliot, F. H., and Platt, B., The effects of insulin, metrazol, and electric shock on blood pyruvate, lactate and glucose. J. Lab. & Clin. Med., 1941, **26**, 1928.

METABOLISM OF PTEROYLGLUTAMIC ACID AND THE CITROVORUM FACTOR IN PATIENTS WITH SCURVY [1, 2]

By GEORGE J. GABUZDA, JR.,[3] GERALD B. PHILLIPS, ROBERT F. SCHILLING, AND CHARLES S. DAVIDSON

(From the Thorndike Memorial Laboratory, Second and Fourth Medical Services [Harvard], Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Mass.)

(Submitted for publication March 26, 1952; accepted June 13, 1952)

The citrovorum factor (CF) is closely related chemically and metabolically to pteroylglutamic acid (PGA) (1–5). CF replaces PGA in the nutrition of certain microorganisms (3, 6) and in some species of animals (6, 7); and also reverses aminopterin activity (3, 7–11). A relationship of CF to PGA is further indicated by the increased urinary excretion of CF which occurs when PGA is fed (12–16). The hematopoietic effect of CF given to patients with pernicious anemia (17–20), or with other nutritional anemias (21, 22), or to monkeys with megaloblastic anemia induced by dietary means (23) also attests to the metabolic activity of this factor.

The synthesis of CF from PGA by rat liver slices is augmented by ascorbic acid (24). This indicates that ascorbic acid may play a role in the conversion of PGA to CF. In order to investigate the role of ascorbic acid in the metabolic relationship of PGA to CF in man, the urinary excretion of CF was determined following PGA administered orally to two patients with scurvy and to a non-scorbutic subject before, during and after ascorbic acid therapy. The results of these studies are presented and indicate that one role of ascorbic acid in man is to facilitate the conversion of PGA to CF.

[1] The expenses of this investigation were defrayed in part by grants from Sharp and Dohme, Inc., West Point, Pennsylvania, and from Merck and Co., Inc., Rahway, New Jersey, to Harvard University.

[2] A preliminary report of this investigation appeared in the Proceedings of the Forty-third Annual Meeting of the American Society for Clinical Investigation held in Atlantic City, New Jersey, April 30, 1951 (J. Clin. Invest., 1951, **30**, 639).

[3] Welch Fellow in Internal Medicine of the National Research Council.

PATIENT MATERIAL AND METHODS

Two men with scurvy and one non-scorbutic male comprise the subjects of this study. The two scorbutics, T. C. age 66, and F. O'D. age 65, were bachelors who lived alone in single rooms, and gave histories of ingesting inadequate quantities of food and of prolonged dietary ascorbic acid deficiency. Clinical evidences of scurvy included multiple perifollicular hemorrhages most prominent on the legs and forearms, ecchymoses especially of the posterior thighs, and hyperkeratotic folliculosis with crinkled, brittle hairs, most apparent on the abdominal wall and thighs. Neither patient showed hypertrophic or bleeding gums. Ascorbic acid was not present in the buffy coat of peripheral venous blood samples obtained from each of these patients. Neither patient had significant anemia. The hemoglobin concentrations were 13.7 gm. and 12.8 gm. per 100 ml., and the red blood cell counts 4.19 million and 4.20 million per cu. mm., respectively. Other blood studies including white blood cell counts, differential count, platelet count, prothrombin time, bleeding time, and coagulation time were within normal limits. A tourniquet test done on a forearm of each of these patients was markedly positive. All of the abnormalities noted were promptly and completely reversed following the administration of ascorbic acid.

The non-scorbutic subject, J. O'C. age 49, was a chronic alcoholic and an epileptic. He had been in the hospital for two months during which time an adequate diet was provided prior to the initiation of the studies described here. At this time physical examination and the usual routine laboratory studies, including tests of liver function, revealed no abnormalities. The peripheral venous buffy coat ascorbic acid content was 44 mg. per cent.

The patients with scurvy were examined shortly after hospital entry and immediately transferred to the Thorndike Metabolic Ward. They did not receive food or medication prior to the institution of the dietary regimen and the studies described. During the entire periods of study these patients were kept at rest in bed and were offered diets consisting of boiled milk and rice, crackers, sugar, coffee, and water. These items of food supplied minimal, if any, quantities of ascorbic acid. The non-scorbutic subject was ambulatory and was maintained on a constant well-balanced dietary regimen pro-

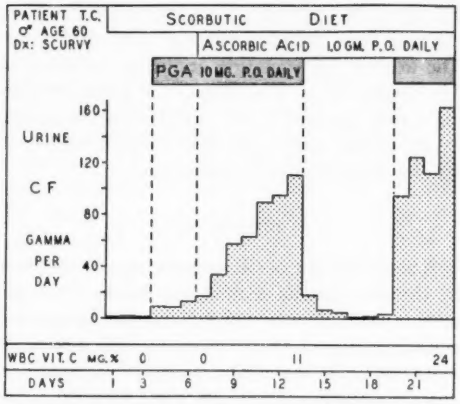

FIG. 1. URINARY EXCRETION OF CF FOLLOWING ORAL PGA IN A PATIENT WITH SCURVY BEFORE AND AFTER ASCORBIC ACID THERAPY

viding 75 gm. of protein and 2,800 calories daily during this study.

The three subjects were observed during initial control periods of three days. Each was then given 10 mg. of PGA [4] orally daily in two equal doses at 9 a.m. and 5 p.m. for from three to six days. During a third period of study of each patient, PGA was continued in the dosage stated and in addition, 1 gm. of ascorbic acid was administered daily for from six to nine days. The ascorbic acid was given orally in the cases of T. C. and J. O'C., and intramuscularly as sodium ascorbate in the case of F. O'D. in four equal doses at 9 a.m., 1 p.m., 5 p.m., and 9 p.m. PGA was then discontinued, but the basal diets and ascorbic acid continued for from six to nine days. In the case of the scorbutic patients, PGA was administered orally on a second occasion according to the same dosage schedule previously employed while ascorbic acid therapy and the basal diets were continued unchanged. Thus, the patients served as their own controls, since observations on the urinary excretion of CF following PGA taken orally were made before and after the alleviation of the scorbutic state by adequate therapy with ascorbic acid.

A final period of study in F. O'D. consisted of the oral supplementation of his diet with 10 mg. of PGA daily for six days without supplemental ascorbic acid. The final period of study in J. O'C. consisted of six days on the constant diet and was therefore identical to the initial study period in this patient.

During these studies the urine specimens were collected and immediately placed in a refrigerator. After completion of each 24-hour urine collection the daily urine volumes were measured, and aliquots stored in a deep freeze at — 20° C. for subsequent microbiologic assay for PGA and CF activity. The microbiologic assays for CF were done according to the method of Sauberlich (1, 12) using a synthetic crystalline material with *L. citrovorum* activity (leucovorin) as the reference standard, and for PGA activity by the growth response of *Lactobacillus casei* (25). Buffy coat ascorbic acid concentrations were measured by the method of Butler and Cushman (26).

[4] The pteroylglutamic acid (folvite) and the crystalline citrovorum factor (leucovorin) used in these studies was generously provided by the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

RESULTS

The results of these studies are presented in Figures 1, 2, and 3. T. C., Figure 1, a patient with scurvy, excreted about 1.2 gamma of urinary CF daily during three initial days of study. When 10 mg. of PGA were administered orally daily for three days, urinary CF excretion ranged from 9.1 to 13.3 gamma daily. One gram of ascorbic acid was then given orally daily for seven days, in addition to PGA. Urinary CF excretion was 16.9 gamma on the first day of ascorbic acid therapy and gradually increased to 110.4 gamma by the seventh day. PGA was then omitted but ascorbic acid continued. Urinary CF returned within four days to initial levels. After six days PGA was given again in addition to ascorbic acid. On this occasion on the first day of PGA administration urinary CF was 93.9 gamma, and averaged 132.6 gamma daily during the three additional days of the final study period.

The second patient with scurvy, F. O'D., Figure 2, excreted about 0.4 gamma of urinary CF

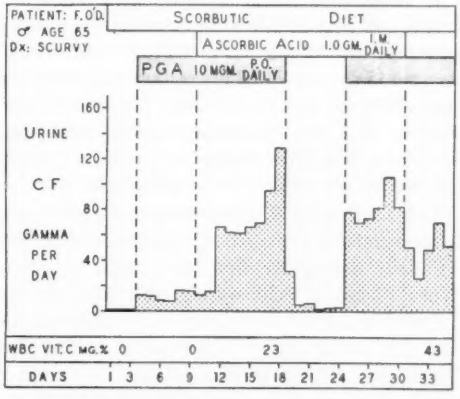

FIG. 2. URINARY EXCRETION OF CF FOLLOWING ORAL PGA IN A PATIENT WITH SCURVY BEFORE AND AFTER ASCORBIC ACID THERAPY

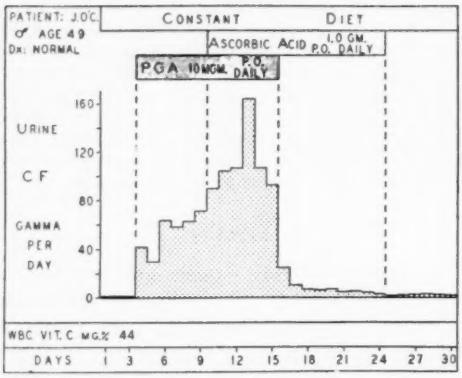

FIG. 3. URINARY EXCRETION OF CF FOLLOWING ORAL PGA IN A NON-SCORBUTIC MALE BEFORE AND AFTER ASCORBIC ACID THERAPY

during the first three days of study. Urinary CF ranged from 7.9 to 16.1 gamma daily when 10 mg. PGA were given orally daily for six days. During the next six days of study, 1.0 gm. of ascorbic acid was given intramuscularly daily to this patient in addition to PGA. Urinary CF was 12.6 gamma on the first day of ascorbic acid therapy, 15.2 gamma on the second day, and ranged from 61.8 to 129.3 gamma daily for four additional days. Ascorbic acid was then continued, but PGA omitted for six days. Urinary CF returned promptly to initial levels. For the next six days, PGA was again given in addition to ascorbic acid. The readministration of PGA was accompanied on the first day of this medication by the urinary excretion of 77.9 gamma of CF, 69.7 gamma on the second day, and 72.5 to 106.0 gamma daily for four additional days of this study period. Finally, ascorbic acid was discontinued, but PGA continued for five days during which time urinary CF excretion averaged 49.3 gamma daily.

J. O'C., Figure 3, a non-scorbutic control patient, excreted approximately 0.9 gamma of urinary CF daily during the first three days of study. When 10 mg. of PGA were given orally, urinary CF excretion rose promptly to 41.8 gamma on the first day and averaged 54.3 gamma daily during the six days of PGA administration. One gram of ascorbic acid was then given orally daily for six days in addition to the PGA. Urinary CF excretion was immediately further increased, and averaged 116.1 gamma daily for this period. Then for nine days, ascorbic acid was continued alone. The urinary CF excretion gradually decreased to 3.0 gamma daily. The urinary CF excretion during a final period of study which was comparable to the initial study period was again very low, ranging from 1.9 to 2.9 gamma daily.

DISCUSSION

A small amount of CF was excreted in the urine by the two patients with scurvy before PGA administration and was slightly increased by the oral administration of PGA before ascorbic acid therapy. However, maximal urinary CF excretion occurred in these patients only when both PGA and ascorbic acid were given. The relatively small increases in urinary CF excretion which were observed in the scorbutic patients when PGA alone was given during the scorbutic state or during the first several days of combined ascorbic acid and PGA therapy, which has also been noted by others (16), is in marked contrast to the immediate and large increases in urinary CF excretion which occurred in these same patients given PGA following adequate therapy with ascorbic acid. A prompt and great increase in urinary CF occurs when PGA is given orally to normal subjects and this increase is further augmented by the administration of ascorbic acid (14, 16). Similar results were obtained in the present study of a non-scorbutic subject during six days of PGA administered orally alone, and then six days of combined PGA and ascorbic acid. Thus the findings in the patients with scurvy given PGA are also markedly different from those observed in the non-scorbutic individual. However, following adequate therapy with ascorbic acid, these patients responded to PGA administration in a manner similar to that observed in the non-scorbutic individual. The failure to observe high urinary CF values in the three subjects studied during those periods of dietary supplementation with ascorbic acid alone indicates that the maximal increases in urine CF excretion observed when both PGA and ascorbic acid were given cannot be attributed to an effect of ascorbic acid upon the constituents of the diets provided.

Large amounts of PGA are known to stimulate the growth of *L. citrovorum* (1). Since the CF

values varied independently from the constant intakes of PGA, it is unlikely that the urinary excretion of PGA in large amounts following its oral administration accounts for the observed alterations in urinary CF excretion. This is obvious from comparing the urine CF values obtained in the patients with scurvy before and during ascorbic acid therapy. Likewise, the urine CF values were not related directly to ascorbic acid administration. However, in order further to validate the microbiologic assay of the urines studied for CF activity content, all urine specimens were assayed for PGA and ascorbic acid content. Although the urines obtained during periods of PGA administration contained very high concentrations of PGA, in no case was this of sufficient magnitude, taking into account the dilution factors used in the assays for CF, to allow for growth stimulation of *L. citrovorum*. Likewise, ascorbic acid alone or in combination with PGA in the concentrations encountered in the urines studied failed to replace CF for the growth of *L. citrovorum*.

The urinary CF excretion pattern observed in patient T. C., Figure 1, might suggest that initial saturation with PGA may be necessary before maximal urinary CF excretion occurs. The urinary PGA excretion data do not, however, indicate an initial retention of PGA. In fact, the quantities of PGA excreted were comparable before and after ascorbic acid therapy. Illustrative data obtained in one of the patients studied are presented in Table I. The failure to observe a gradually increasing CF excretion in patient F. O'D., Figure 2, during six days of PGA administration before ascorbic acid therapy also makes it unlikely that the gradually increasing urinary CF excretion noted in patient T. C., Figure 1, would have occurred even if ascorbic acid had not been given.

The results of the PGA assays on the urines also demonstrate that the failure of maximal urinary CF excretion to occur in the scorbutic state is not related to a failure of absorption of the orally administered PGA in the absence of ascorbic acid since in both patients with scurvy, large amounts of urinary PGA were excreted daily before ascorbic acid therapy (Table I).

The relatively small but significant increase in urinary CF excretion noted in the patients with scurvy given PGA orally is not readily explained.

TABLE I

Urinary PGA and CF excretion following oral PGA before and during ascorbic acid therapy in a patient with scurvy (T. C.)

Day of study	Oral medication		Urinary excretion	
	PGA	Ascorbic acid	PGA	CF
	mg.	*gm.*	*γ*	*γ*
1			8.0	1.1
2			4.4	1.3
3			6.9	1.1
4	10.0		5,400	9.1
5	10.0		5,500	8.9
6	10.0		7,200	13.3
7	10.0	1.0	8,400	16.9
8	10.0	1.0	8,500	33.4
9	10.0	1.0	6,800	57.6
10	10.0	1.0	5,600	62.4
11	10.0	1.0	6,600	89.1
12	10.0	1.0	6,800	94.5
13	10.0	1.0	5,900	110.4
14		1.0	190	18.1
15		1.0	15.7	6.2
16		1.0	10.2	4.6
17		1.0	2.7	0.9
18		1.0	3.7	1.2
19		1.0	8.4	3.6
20	10.0	1.0	6,600	93.9
21	10.0	1.0	6,100	123.9
22	10.0	1.0	8,000	111.4
23	10.0	1.0	8,100	162.5

This may be related to the large doses of PGA used. Other possibilities are that although ascorbic acid was not found in the buffy coats of the patients studied, they were not totally deficient in ascorbic acid, or perhaps that reducing substances other than ascorbic acid may contribute to the biosynthesis of CF from PGA. In this regard, glucoascorbic acid as well as ascorbic acid markedly increased the urinary excretion of CF in rats given PGA, while cysteine and glutathione were without effect (16).

Urinary CF did not increase promptly in the patients with scurvy after ascorbic acid was given. Several days of ascorbic acid therapy were required before outputs of CF comparable to those observed in normal individuals occurred. This delay in CF excretion may be related to the quantity of ascorbic acid required to restore adequate tissue concentrations of this substance, or it may indicate tissue storage of CF occurring before excretion of this material commences.

The results of these studies indicate that PGA is closely related metabolically to CF in adult man, in confirmation of similar findings by others (12–16). In addition, the data presented indicate that

ascorbic acid greatly facilitates the conversion of PGA to CF, and suggest that one of the biochemical functions of ascorbic acid in man is to take part in the biosynthesis of CF from PGA.

SUMMARY AND CONCLUSIONS

The urinary excretion of CF was determined microbiologically following PGA administered orally to two men with scurvy and to one non-scorbutic adult before, during and after ascorbic acid therapy.

A small amount of CF was excreted by the patients with scurvy and by the non-scorbutic subject during the initial control period. Urinary CF was increased slightly when PGA was administered orally to the patients during the scorbutic state. However, maximal CF excretion, comparable to that observed in the non-scorbutic individual, occurred in the patients with scurvy only after adequate therapy with ascorbic acid.

The data indicate that CF is metabolically related to PGA, and that one role of ascorbic acid in man is to provide for the conversion of PGA to CF.

ACKNOWLEDGMENTS

The authors wish to thank the Misses Alice N. Ballou, Leonora De Carli, Elaine Hirschberg, and Virginia Olsson for technical assistance.

REFERENCES

1. Sauberlich, H. E., and Baumann, C. A., A factor required for the growth of Leuconostoc citrovorum. J. Biol. Chem., 1948, **176**, 165.
2. Sauberlich, H. E., and Baumann, C. A., Further studies on the factor required by Leuconostoc citrovorum 8081. J. Biol. Chem., 1949, **181**, 871.
3. Broquist, H. P., Stokstad, E. L. R., and Jukes, T. H., Some biological and chemical properties of the citrovorum factor. J. Biol. Chem., 1950, **185**, 399.
4. Shive, W., Bardos, T. J., Bond, T. J., and Rogers, L. L., Synthetic members of the folinic acid group. J. Am. Chem. Soc., 1950, **72**, 2817.
5. Brockman, J. A., Jr., Roth, B., Broquist, H. P., Hultquist, M. E., Smith, J. M., Jr., Fahrenbach, M. J., Cosulich, D. B., Parker, R. P., Stokstad, E. L. R., and Jukes, T. H., Synthesis and isolation of a crystalline substance with the properties of a new B vitamin. J. Am. Chem. Soc., 1950, **72**, 4325.
6. Broquist, H. P., Stokstad, E. L. R., and Jukes, T. H., Comparative biological activity of crystalline citrovorum factor and pteroylglutamic acid. Federation Proc., 1951, **10**, 167.
7. Waisman, H. A., Green, M., Munoz, J. C., Ramenchik, A., and Richmond, J. B., Role of aureomycin and citrovorum factor in "folic acid" deficiencies. Proc. Soc. Exper. Biol. & Med., 1951, **76**, 384.
8. Nichol, C. A., and Welch, A. D., On the mechanism of action of aminopterin. Proc. Soc. Exper. Biol. & Med., 1950, **74**, 403.
9. Schoenbach, E. B., Greenspan, E. M., and Colsky, J., Reversal of aminopterin and amethopterin toxicity by citrovorum factor. J. A. M. A., 1950, **144**, 1558.
10. Burchenal, J. H., Babcock, G. M., Broquist, H. P., and Jukes, T. H., Prevention of chemotherapeutic effects of 4-amino-N^{10}-methyl-pteroylglutamic acid on mouse leukemia by citrovorum factor. Proc. Soc. Exper. Biol. & Med., 1950, **74**, 735.
11. Cravens, W. W., and Snell, E. E., Reversal of aminopterin inhibition in the chick embryo with the Leuconostoc citrovorum factor. Proc. Soc. Exper. Biol. & Med., 1950, **75**, 43.
12. Sauberlich, H. E., The effect of folic acid upon the urinary excretion of the growth factor required by Leuconostoc citrovorum. J. Biol. Chem., 1949, **181**, 467.
13. Anker, R. M., Boehne, J. W., and Welch, A. D., A folic acid-derived growth factor for Leuconostoc citrovorum in human urine. Federation Proc., 1950, **9**, 351.
14. Broquist, H. P., Stokstad, E. L. R., and Jukes, T. H., Biochemical studies with the "citrovorum factor." J. Lab. & Clin. Med., 1951, **38**, 95.
15. Welch, A. D., Nichol, C. A., Anker, R., and Boehne, W., Urinary excretion of a biologically active metabolic alteration product derived from folic acid. J. Pharm. & Exper. Therap., 1951, **101**, 37.
16. Welch, A. D., Nichol, C. A., Anker, R. M., and Boehne, J. W., III, The effect of ascorbic acid on the urinary excretion of citrovorum factor derived from folic acid. J. Pharm. & Exper. Therap., 1951, **102–3**, 403.
17. Meyer, L. M., Brahin, C. M., and Sawitsky, A., Treatment of pernicious anemia with citrovorum factor. Proc. Soc. Exper. Biol. & Med., 1951, **76**, 86.
18. Ellison, R. R., Wolfe, S., Lichtman, H., Ginsberg, V., and Watson, J., Effect of citrovorum factor in pernicious anemia. Proc. Soc. Exper. Biol. & Med., 1951, **76**, 366.
19. Jarrold, T., Horrigan, D., Thompson, C., and Vilter, R. W., The hematologic effect of folinic acid (citrovorum factor) in persons with pernicious anemia. Science, 1951, **113**, 688.
20. Davidson, L. S. P., and Girdwood, R. H., Treatment of pernicious anemia with leuconostoc citrovorum factor. Lancet, 1951, **1**, 722.
21. Spies, T. D., Lopez, G. G., Milanes, F., Toca, R. L., Reberedo, A., and Stone, R. E., The response of patients with pernicious anemia with nutritional macrocytic anemia and with tropical sprue to folinic

acid or citrovorum factor. South. M. J., 1950, **43**, 1076.

22. Woodruff, C. W., Peterson, J. C., and Darby, W. J., Citrovorum factor and folic acid in treatment of megaloblastic anemia in infancy. Proc. Soc. Exper. Biol. & Med., 1951, **77**, 16.
23. May, C., Sundberg, R. D., and Scharr, F., Comparison of effects of folic acid and folinic acid in experimental megaloblastic anemia. J. Lab. & Clin. Med., 1950, **36**, 963.
24. Nichol, C. A., and Welch, A. D., Synthesis of citrovorum factor from folic acid by liver slices; augmentation by ascorbic acid. Proc. Soc. Exper. Biol. & Med., 1950, **74**, 52.
25. Teply, L. J., and Elvehjem, C. A., The titrimetric determination of "Lactobacillus casei factor" and "folic acid." J. Biol. Chem., 1945, **157**, 303.
26. Butler, A., and Cushman, M., Distribution of ascorbic acid in the blood and its nutritional significance. J. Clin. Invest., 1940, **19**, 459.

CARDIAC FAILURE SECONDARY TO INEFFECTIVE BELLOWS ACTION OF THE CHEST CAGE

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(Submitted for publication April 7, 1952; accepted June 13, 1952)

Baldwin, Cournand, and Richards (1) have described the following classification of pulmonary insufficiency:

1. Ventilatory
   a. Restrictive, *viz.*, pulmonary fibrosis, kyphoscoliosis.
   b. Obstructive, *viz.*, pulmonary emphysema.
2. Alveolar-respiratory
   a. Distributive, *viz.*, pulmonary emphysema.
   b. Diffusional, *viz.*, pulmonary scleroderma.

The two cases to be presented demonstrate ventilatory insufficiency of the restrictive type due in the first instance to primary neuromuscular dysfunction and in the second to chronic pleural disease with resulting calcification and restriction of the underlying parenchyma and diaphragm. Of particular interest was the development of congestive heart failure in both of these patients. These cases represent examples of cardiac failure secondary to an ineffective bellows action of the chest cage without significant intrinsic pulmonary disease.

CASE REPORTS

Case 1

This 34 year old white male was well until 1945 when he noted the gradual onset of weakness of the right side of the face. A few months later he began to have bilateral jaw weakness, and difficulty in swallowing food with regurgitation of fluid through the nose. In 1947 there was onset of occasional diplopia. He was admitted to this hospital on three different occasions between January 1947 and November 1948 and on all these admissions showed signs and symptoms of bulbar involvement. These signs and symptoms persisted with little change except for development of dysarthria. In November 1949 he complained of difficulty in maintaining erect posture and he was readmitted for his fourth and final admission. At this time the patient stated he had noted dyspnea on exertion for the preceding one and a half years and during the month before admission, cyanosis of the lips had become apparent.

On admission the patient appeared chronically ill without respiratory distress but with cyanosis of the lips, nailbeds, and skin. There was a marked dysarthria. His chest cage moved very little on normal respiration but he had good expansion with effort and could clear most of his cyanosis with forced breathing. The heart was not remarkable on examination except for a markedly accentuated P2. The lungs were clear to percussion and auscultation. The abdominal examination was within normal limits. Neurological examination revealed the right pupil to be larger than the left, and both reacted sluggishly to light and accommodation. The external ocular movements were normal but he had diplopia in all fields. The disc margins were slightly blurred nasally and he had small hemorrhages about the discs. His gait was normal. There was weakness of the jaw, right facial, palatal, tongue, neck, and shoulder muscles with fasciculations noted in these groups. In addition there was atrophy of the masseters, platysmae, tongue, neck and shoulder muscles. There were a few fasciculations of the triceps muscles but no weakness or atrophy. The deep tendon reflexes of the arms were two to three plus bilaterally and there was greater finger stretch on the left than on the right. The abdominal reflexes were diminished bilaterally. There was no atrophy or weakness of the legs and the sensory examination was normal.

Laboratory examinations including complete blood count, urine analysis, blood chemistries, spinal fluid, serology, chest X-ray, skull plates, and electrocardiographs were all within normal limits.

The patient's neurological status remained unchanged while under observation and adequate nutrition was maintained with tube feeding. Two months after admission a medical consultation was requested because of increasing cyanosis. At this time marked cyanosis was observed without respiratory distress. Expansion of the chest cage during ordinary breathing was minimal but with forced inspiration he was capable of a three inch chest expansion. The lungs were clear and examination of the heart was unremarkable except for an accentuated second pulmonic sound. Abdominal examination was negative. There was no peripheral edema or venous engorgement. The red blood count was 6.75 million with a hemoglobin of 19.4 grams and a hematocrit of 64%. Fluoroscopic examination revealed the trachea to be in the midline and the motion of the bony framework was good. There was normal radiolucency of the lung fields. The range of motion of the right diaphragm was one

inch; that of the left diaphragm one-half inch. There was no diaphragmatic paralysis. The lung fields were clear. Examination of the heart revealed slight left ventricular rounding, moderate right ventricular enlargement, and marked enlargement of the pulmonary conus. The electrocardiograph demonstrated evidence of right ventricular hypertrophy. Venous pressure was 180 mm. of citrate and decholin circulation time 28 seconds. Because of these findings, the patient was digitalized. There was a five pound diuresis, and the venous pressure and circulation time returned to normal. Following this the patient was studied in the cardiopulmonary laboratory (Table I). Early in June 1950, despite vigorous therapy, he went into intractable congestive heart failure and died.

Autopsy examination revealed the following pertinent findings. There was clubbing of the fingers and moderate subcutaneous edema. The lungs showed a moderate degree of congestion with occasional scattered areas of chronic pneumonitis in the lower lobes, probably on an aspirational basis. There was no gross or microscopic evidence of significant emphysema or fibrosis. The heart was enlarged weighing 500 grams. The coronary arteries, myocardium, endocardium and valvular leaflets were normal. The right ventricular chamber was dilated and the right ventricular wall hypertrophied measuring 1 cm. in thickness. The pulmonic valve ring was dilated measuring 10 cm. in circumference. The right auricle was slightly dilated as was the tricuspid valve ring which measured 14.5 cm. in circumference. The left atrium and mitral valve were normal. The left ventricular wall was slightly hypertrophied to approximately 1.5 cm. in thickness. The aortic valve was normal in size and appearance. The pulmonary arteries showed mild atherosclerotic changes. The gastrointestinal tract, biliary system, genitourinary tract, spleen, pancreas, adrenals, thyroid, lymph nodes, and skeletal system were all normal. There was chronic passive congestion of the liver, spleen and kidneys. The liver weighed 1,750 grams and the spleen 220 grams. The central nervous system, including brain and spinal cord, showed no distinctive lesions on multiple sections with different staining techniques including Mahon's. The peripheral nerves were normal.

Despite the negative autopsy findings, it was the opin-

TABLE I

RESULTS OF PULMONARY FUNCTION STUDIES IN CASES 1 AND 2

		Case 1		
I	Lung Volumes	Predicted		Observed
	Vital Capacity (lying) c.c.	4770		2285
	Residual air, c.c.	1180		1005
	Total capacity, c.c.	5950		3680
	R.A./T.C. %	20		27
	Alv. N_2 %	<2.5		1.77
II.	Maximum Breathing Capacity, L/min	119		38
III.	Basal Ventilation/min L/M^2 B.S.A.	3.20 ± 0.65		3.33
IV.	Respiratory Gases in Arterial Blood	Rest	Rest	Exerc.
	CO_2 content vols. %	48.5 ± 4.5	64.4	72.4
	O_2 saturation %	96 ± 2	73.3	65.5
		Room air		
	pCO_2 mm. Hg.	40	84	
	pO_2 mm. Hg.	95	49	
	pH	7.4	7.2 (calc.)	
V.	Calculated Alveolar-Arterial Gradient on Room Air	0 to 12	Normal	

TABLE I (Continued)

CASE 2

		Predicted	Observed
I.	Lung Volumes		
	Vital Capacity (lying) c.c.	3800	900*
	Residual air, c.c.	1220	880
	Total Capacity, c.c.	5020	1690*
	R.A./T.C. %	24	42
	Alv. N_2 %	less than 2.5	less than 2.5
II.	Maximum Breathing Capacity L/min.	90	38*

*Results inaccurate because of poor cooperation.

		Rest		Exerc.	
		Predicted	Observed	Predicted	Observed
III.	Ventilation/min. L/M^2 B.S.A.	3.20 ± 0.65	6.45	8.70 ± 0.81	12.98
	O_2 Intake in cc/M^2 B.S.A.	129 ± 13	161	480 ± 74	390
	O_2 Removal in cc/L ventilation	46.8 ± 7.1	30.4	54.8 ± 6.2	36.8

		Rest		
		Predicted	Observed	Recovery
IV.	Respiratory Gases in Arterial Blood			
	CO_2 content vols. %	48.5 ± 4.5	58.3	54.7
	O_2 saturation %	96 ± 2	92.3	84.6
	pCO_2 mm. Hg.	40	69	
	pO_2 mm. Hg.	95	66	
	pH	7.4		

V.		Alv. pO_2 mm. Hg.	70
		Art. pO_2 mm. Hg.	66
	Room air breathing	Art. pCO_2 mm. Hg.	69
		Alveolar-arterial gradient	4
		Dead space ml.	193
		Dead space % tidal air	56
		ph calculated	7.27
		Alv. pO_2	118
		Art. pO_2	105
	High oxygen breathing (29.25%)	Alveolar-arterial gradient	13
		Dead space ml.	178
		Dead space % tidal air	55
		ph calculated	7.27

ion of the neurological service that this patient's clinical picture could best be explained by ascribing the changes to amyotrophic lateral sclerosis.

Case 2

A 56 year old Puerto Rican male was admitted to the hospital because of increased dyspnea and orthopnea for three weeks, and a non-productive cough for a few days. In 1921 and 1925 the patient had "pleurisy." In 1936 he was told that he had "calcifications in his chest." In 1946 he was refused employment because of an abnormal chest X-ray. Three years before admission there was an episode of wheezing and undue shortness of breath which lasted a few days and then ceased spontaneously. During the two years prior to admission there was ankle edema. Because of the increase in dyspnea the patient sought hospitalization.

At the time of admission the patient's temperature was 103° F., pulse 116. He was orthopneic and cyanotic. Examination of the chest disclosed poor expansion bilaterally with more limitation on the right. There was dullness over the lower half of the right lung field and at the left base with diminished breath sounds and tactile fremitus over these areas. There were moist rales at the left base with occasional wheezing over the right upper lung field. The second pulmonic sound was accentuated. The liver edge was two finger breadths below the right costal margin, and tender. There was two plus pretibial edema.

The blood count on admission revealed a normal white blood count and differential. The red blood count was 6.0 million, hemoglobin 18.5 grams, and the hematocrit was 62%. An X-ray of the chest disclosed extensive calcification surrounding the lower two-thirds of both lung

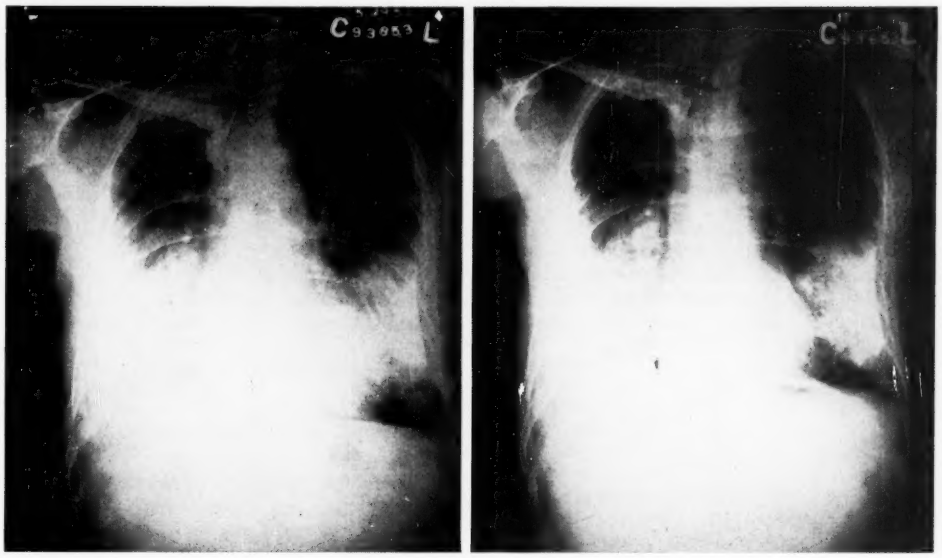

FIG. 1A. CASE 2 DEMONSTRATING CALCIFIED PLEURITIS AND CARDIAC ENLARGMENT BEFORE THERAPY
FIG. 1B. AFTER THERAPY DEMONSTRATING RETURN OF HEART SIZE TO NORMAL

fields and an increase in the transverse diameter of the heart (Figure 1A). An electrocardiogram was consistent with right ventricular hypertrophy. The venous pressure on admission was 280 mm. of citrate.

The patient was treated with penicillin and bed rest for the first five days with no change in the clinical course. On the fifth hospital day the patient was digitalized, placed on a low salt diet, and oxygen was administered intermittently by mask. Later that day he became semistuporous. He was placed in an oxygen tent and given mercurial diuretics. Shortly thereafter he became comatose although the cyanosis diminished. The stupor per-

TABLE II

	pO_2 mm. Hg.	O_2 Capac. vols. %	O_2 Sat. %	pCO_2 mm. Hg.	CO_2 cont. vols. %	ph
6/1/51 Room air immediately after oxygen tent with narcosis	26	22.2	39.2	96	59.3	7.14
6/4/51 Breathing room air	55	20.1	87.6	57	62.4	7.40
6/6/51 Respirator with nasal oxygen	98	19.7	98.9	68	61.1	7.30
6/12/51 Respirator with nasal oxygen	147*	20.2	100	51	59.4	7.42

*Inaccurate, above range of applicability of bubble technic.

sisted until the patient was removed from the oxygen tent for routine care the following day. Arterial blood studies suggested that carbon dioxide narcosis was responsible for the coma (Table II). In order to combat the CO_2 narcosis, artificial respiration with the Pneumatic Balance Resuscitator was initiated. Within a short time the patient was more alert and there was no cyanosis. A phlebotomy was also performed. Artificial respiration was discontinued later that day, and the patient maintained satisfactory clinical progress for the next 48 hours. The blood gas studies reflected this improvement. Although the patient had improved considerably, oxygen therapy was again administered because of the anoxia disclosed by the arterial blood studies. Artificial respiration was reinstituted by means of a Drinker-Collins respirator, and oxygen was administered by nasal catheter. This was continued for one week along with penicillin, bronchodilators and continued digitalis therapy, phlebotomy, low salt diet, and occasional mercurial diuretics. At the end of this period the patient was relatively asymptomatic and the signs of congestive heart failure had disappeared. Follow-up X-rays disclosed that the heart size had returned to normal (Figure 1B). Fluoroscopy revealed the pleural calcifications, no motion of the rib cage, and restriction of diaphragmatic motion. Complete pulmonary function studies were performed one month after recovery from the acute episode (Table I). Three months after discharge the patient was seen in the Follow-up Clinic. He was asymptomatic and capable of climbing two flights of stairs. Digitalis therapy and a low salt diet were continued.

METHODS

The measurement of residual air volume was done using an open circuit method in which the nitrogen of the lungs is washed out by continuous inhalation of pure oxygen and collected over a period of seven minutes. An index of intrapulmonary mixing was obtained by sampling of the alveolar air at the end of this period (1).

The measurements of lung volumes and maximum breathing capacity were obtained using the spirographic technique (1).

Arterial blood samples were obtained from the brachial artery using an indwelling Cournand-type needle. The blood was collected by the technique described by Riley, Proemmel and Franke (2), using heparin to prevent clotting and a small globule of mercury in the syringe to facilitate mixing. The oxygen content and capacity and the carbon dioxide content were determined on duplicate samples using the Van Slyke-Neill apparatus (3). All samples checked within 0.2 volume %.

The arterial blood tensions of oxygen and of carbon dioxide were determined directly in mm. of mercury, using the direct method of Riley (2). Duplicate measurements were made using two Roughton-Scholander syringes, and all samples checked within 2 mm. of mercury. Expired air samples were collected in duplicate simultaneously with the blood specimens. Oxygen intake and carbon dioxide output were calculated from the percentage of oxygen and carbon dioxide found in these expired air samples, as determined in a Scholander gas analyzer (4). The duplicate measurements checked within 0.04%.

Arterial blood samples and expired air were collected under basal conditions with the patients breathing room air, high, and low oxygen concentrations, each for twenty minute periods when indicated. Finally oxygen saturation was determined after one minute of standard exercise and the expired gas during the exercise was collected in a Douglas bag and analyzed. During the five minute recovery period the expired air was collected and analyzed. The expired air, except during exercise, was collected in the Tissot apparatus and the spirogram attached permitted calculation of the respiratory rate and tidal air under the various conditions. The alveolar-arterial gradients were calculated from the data obtained according to the method of Lilienthal, Riley, Proemmel and Franke (5).

Values for pH were calculated from line charts of the Henderson-Hasselbalch equation (6).

COMMENTS

Case 1 disclosed a markedly reduced vital capacity, total capacity and maximum breathing capacity. The residual air and alveolar nitrogen after breathing pure oxygen for seven minutes were normal. The ratio of residual air to total capacity was slightly but not significantly increased and reflected the decrease in total capacity. Thus the lung volume studies revealed principally a reduction in vital capacity which was due primarily to the marked limitation of diaphragmatic excursion. The normal residual air and alveolar nitrogen indicated the absence of any significant degree of emphysema. During the performance of the maximum breathing capacity an element of fatigue was apparent in that the patient was unable to maintain the depth or speed of ventilation uniformly for the 12 second interval. This was probably due to muscle anoxia and weakness associated with chronic disease. In addition, a satisfactory depth of respiration could not be established presumably as a result of impaired diaphragmatic excursion. Finally an adequate speed of ventilation could not be established because of muscular weakness and poor coordination. The total basal ventilation was normal but the effective alveolar ventilation was grossly inadequate as reflected by the high arterial pCO_2. Despite the presence of strong stimuli for hyperventilation (high arterial pCO_2 and low arterial pH and pO_2) the resting ventilation was normal. This would suggest decreased sensitivity

of the respiratory center to chemical stimuli. In addition, the polycythemia may have been an aggravating factor (7). The inadequate alveolar ventilation resulted from the fact that too large a proportion of the total ventilation was wasted in ventilating the dead space as a result of the shallow breathing. It is of interest to note that this patient's resting ventilation was less than 10% of his maximum breathing capacity which would account for the absence of dyspnea at rest in the presence of severe anoxemia (1). The arterial CO_2 content was elevated but not commensurate with the pCO_2 so that the pH was decreased. The arterial pO_2 and oxygen saturation were markedly reduced and the latter decreased even further after standard exercise. These changes were due solely to inadequate alveolar ventilation. The calculated alveolar-arterial gradient on room air, assuming an RQ of 0.8 to 0.9, was normal. Because of the patient's muscle weakness he was unable to keep a mouthpiece in place for prolonged periods of time, and the RQ of 0.8 to 0.9 was therefore assumed.

Case 2 demonstrated marked decrease in all lung volumes. The observed figures for the vital capacity, total capacity and maximum breathing capacity probably were inaccurate since the patient did not appear to cooperate fully during the performance of these tests. The ratio of residual air to total capacity was high due to the fact that the total capacity reduction was out of proportion to that of the residual air. The maximum breathing capacity was reduced. These changes were primarily due to the chronic constrictive calcific pleuritis which interfered with the bellows action of the chest cage. The high ratio of residual air to total capacity was not a reflection of emphysema since the alveolar nitrogen was less than 2.5%. The tracings showed no graphic evidence of a prolonged expiratory phase. There was no evidence for impairment of gas exchange. Ventilation studies demonstrated that the patient was hyperventilating at rest and during exercise, presumably as a response to the high arterial pCO_2 and anoxia. This patient's calcific constrictive pleuritis markedly reduced his effective alveolar ventilation. Thus, his respirations were rapid but shallow and probably did little more than wash out the dead space. This caused a high arterial pCO_2 and CO_2 content and anoxia. There was no indication of significant abnormalities of gas exchange since the gradient studies were within normal limits (5, 8).

The patient was admitted with an acute pulmonary infection. This was enough to accentuate the anoxia and thereby increase the pulmonary hypertension and precipitate cardiac failure. He was treated with antibiotics, bronchodilators, digitalis, phlebotomy, and oxygen. The use of continuous oxygen therapy in patients with chronic anoxia has been known to produce CO_2 narcosis (9–13) and this patient was a vivid example of this phenomenon (Table II). The Pneumatic Balance Resuscitator probably represented a life saving procedure at this time. The use of the respirator permitted continuous use of oxygen therapy without the threat of the return of CO_2 narcosis. This will be discussed in another paper (14).

DISCUSSION

We have presented two patients whose primary difficulty was a defective bellows action of the chest cage. In the first case this was due to disease of the neuromuscular system. In the second case the patient had extensive calcific pleuritis of undetermined etiology which interfered with the mobility of the thoracic wall. In the latter instance there undoubtedly existed some degree of pulmonary

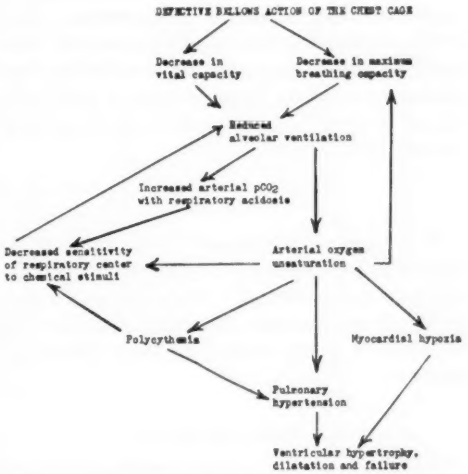
DEFECTIVE BELLOWS ACTION OF THE CHEST CAGE
Decrease in vital capacity
Decrease in maximum breathing capacity
Reduced alveolar ventilation
Increased arterial pCO2 with respiratory acidosis
Decreased sensitivity of respiratory center to chemical stimuli
Arterial oxygen unsaturation
Polycythemia
Myocardial hypoxia
Pulmonary hypertension
Ventricular hypertrophy, dilatation and failure

FIG. 2. SEQUENCE OF EVENTS WHICH MAY HAVE OCCURRED IN TWO CASES WITH AN INEFFECTIVE BELLOWS ACTION OF THE CHEST CAGE

disease, probably on a tuberculous basis. In view of the entire clinical picture and pulmonary function studies, we are inclined to believe that parenchymal disease contributed little if any to the abnormalities observed.

In both these patients (Figure 2) the defective bellows action resulted in a reduction in vital capacity, maximum breathing capacity, and ineffective alveolar ventilation manifested by a high arterial pCO_2 and arterial oxygen unsaturation. The anoxia was probably a stimulus for the production of the polycythemia (15). The hypercapnia, anoxia, and polycythemia all represented factors favoring medullary center damage which these patients demonstrated. There is evidence to indicate that both polycythemia (7) and anoxia (16, 17) may lead to pulmonary hypertension and eventually cardiac failure which these patients also manifested. In both these patients the normal changes in circulatory dynamics associated with the mechanics of respiration were undoubtedly altered (18). No objective studies were made to evaluate these factors.

Finally, it is of interest to correlate the situation in primary polycythemia with the sequence of events in these two cases. In the former instance there is polycythemia which we believe may lead to hypoxemia (7). In the latter we have ineffective bellows action of the chest cage leading to hypoxemia and secondary polycythemia. Evidently, once polycythemia and hypoxemia have occurred, the subsequent tendency to develop congestive heart failure probably depends upon similar mechanisms, *viz.*, myocardial hypoxia and pulmonary hypertension.

SUMMARY

Two cases of ventilatory insufficiency of the restrictive type resulting in congestive heart failure have been presented. There was no evidence of significant intrinsic pulmonary disease. The sequence of events leading to heart failure are discussed.

ACKNOWLEDGMENTS

The authors wish to acknowledge their appreciation to Dr. Richard L. Riley for his many helpful comments in the preparation of this manuscript, and to Mrs. Blanche Devlin for her invaluable aid in the laboratory procedures.

REFERENCES

1. Baldwin, E. de F., Cournand, A., and Richards, D. W., Jr., Pulmonary insufficiency. I. Physiological classification, clinical methods of analysis, standard values in normal subjects. Medicine, 1948, **27**, 243.
2. Riley, R. L., Proemmel, D. D., and Franke, R. E., A direct method for determination of oxygen and carbon dioxide tensions in blood. J. Biol. Chem., 1945, **161**, 621.
3. Van Slyke, D. D., and Neill, J. M., The determination of gases in blood and other solutions by vacuum extraction and manometric measurements. I. J. Biol. Chem., 1924, **61**, 523.
4. Scholander, P. F., Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. J. Biol. Chem., 1947, **167**, 235.
5. Lilienthal, J. L., Jr., Riley, R. L., Proemmel, D. D., and Franke, R. E., An experimental analysis in man of the oxygen pressure gradient from alveolar air to arterial blood during rest and exercise at sea level and altitude. Am. J. Physiol., 1946, **147**, 199.
6. Van Slyke, D. D., and Sendroy, J., Jr., Studies of gas and electrolyte equilibria in blood. XV. Line charts for graphic calculations by the Henderson-Hasselbalch equation, and for calculating plasma carbon dioxide content from whole blood content. J. Biol. Chem., 1928, **79**, 781.
7. Newman, W., Feltman, J. A., and Devlin, B., Pulmonary function studies in polycythemia vera. Am. J. Med., 1951, **11**, 706.
8. Rahn, H., Effects of unequal blood flow and ventilation upon the alveolar-arterial gradient. Federation Proc., 1949, **8**, 129.
9. Barach, A. L., and Richards, D. W., Jr., Effects of treatment with oxygen in cardiac failure. Arch. Int. Med., 1931, **48**, 325.
10. Barach, A. L., Effect of low and high oxygen tensions on mental functioning. J. Aviation Med., 1941, **12**, 30.
11. Comroe, J. H., Jr., Bahnson, E. R., and Coates, E. O., Jr., Mental changes occurring in chronically anoxemic patients during oxygen therapy. J. A. M. A., 1950, **143**, 1044.
12. Davies, C. E., and Mackinnon, J., Neurological effects of oxygen in chronic cor pulmonale. Lancet, 1949, **2**, 883.
13. Donald, K., Neurological effects of oxygen. Lancet, 1949, **2**, 1056.
14. Stone, D. J., Schwartz, A., Newman, W., Feltman, J. A., and Lovelock, F. J., The precipitation by pulmonary infection of anoxia, cardiac failure, and respiratory acidosis: pathogenesis and treatment. Am. J. Med., to be published.

15. Viault, Sur la quantité d'oxygène contenue dans le sang des animaux des hauts plateaux de l'Amérique du Sud. Compt. rend. Acad. Sc., 1891, **112**, 295.
16. Motley, H. L., Cournand, A., Werko, L., Himmelstein, A., and Dresdale, D., The influence of short periods of induced acute anoxia upon pulmonary artery pressures in man. Am. J. Physiol., 1947, **150**, 315.
17. Westcott, R. N., Fowler, N. O., Scott, R. C., Hauenstein, V. D., and McGuire, J., Anoxia and human pulmonary vascular resistance. J. Clin. Invest., 1951, **30**, 957.
18. Lawson, H. D., Bloomfield, R. A., and Cournand, A., The influence of the respiration on the circulation in man. Am. J. Med., 1946, **1**, 315.

EFFECTS OF ACUTE ANOXIA ON THE CIRCULATION AND RESPIRATION IN PATIENTS WITH CHRONIC PULMONARY DISEASE STUDIED DURING THE "STEADY STATE" [1]

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(Submitted for publication May 10, 1952; accepted June 13, 1952)

INTRODUCTION

The effect of acutely induced anoxia upon the respiration and circulation of man and animals has been repeatedly investigated (1–4). Various circulatory responses have been described, and it is apparent that the observations of different investigators have sometimes been divergent. For example, following the induction of acute anoxia, the minute output of the heart has been noted by individual authors to increase, decrease, or remain unchanged. Grollman (1) reviewed these results and ascribed them to several readily discernible causes: *1*) inaccuracies inherent in the methods used to measure cardiac output; *2*) failure to distinguish between the physiologic responses of anesthetized versus unanesthetized animals; *3*) the variety of species studied, and *4*) dissimilar degrees of anoxia.

A previous report from this laboratory on the effects of acute anoxia on pulmonary artery pressure (3) included measurements of the cardiac output using the "Direct Fick Method" in five normal subjects. It was found in these studies that short periods of anoxia (breathing 10 per cent oxygen for approximately 10 minutes) usually resulted in a decrease of the estimated cardiac output. This response to anoxia which has been observed in normal individuals, merits further consideration in patients with chronic pulmonary disease where spontaneous variations in anoxia and cardiac output incident to their daily activity may contribute to the evolution of cardiopulmonary disease.

It is the purpose of this report to analyze the effects of breathing gas mixtures with various oxygen concentrations upon the circulation of 35 patients with chronic pulmonary disease. Because a "steady state" of the respiration and circulation is essential when one measures cardiac output during cardiac catheterization by the Fick principle, a special attempt has been made to include only studies in which such a state was attained and maintained during the period of observation.

METHODS

All patients were studied in the unanesthetized, postabsorptive, "basal" state. The observations were usually begun approximately one-half hour after arrival in the laboratory, and considerable attention was given to the achievement of complete relaxation during the period of observation. This was facilitated by respiratory measurements and arterial puncture on previous days which served to familiarize the patient with the laboratory, its apparatus and personnel. In some, cardiac catheterization had been previously performed. All determinations were completed within two hours after placement of the cardiac catheter. The methods used were identical with those previously described from this laboratory (5, 6).

The heart rate was observed on the electrocardiogram throughout the entire procedure, including the period of cardiac catheterization and arterial puncture. Lability of heart rate while the subject was at rest and breathing ambient air, supplemented other clinical guides to sympathetic overactivity (tachypnea, moist skin, wide pupils).

The initial series of determinations of cardiac output and blood pressures (pulmonary artery and brachial artery) were made while the relaxed subject was breathing 21 per cent oxygen. After expired gas, mixed venous blood and arterial blood had been simultaneously collected to measure the cardiac output, the entire procedure was repeated with the subject inspiring a higher (if original

[1] This investigation was supported (in part) by a research grant (PHS Grant H-833[C]) from the National Heart Institute of the National Institutes of Health, Public Health Service, with additional support from the Life Insurance Medical Research Fund and the American Heart Association.

[2] Established Investigator of the American Heart Association.

[3] James Alexander Miller Research Fellow.

arterial oxygen saturation was less than 90 per cent), or lower oxygen mixture. The blood and expired gas samples were also used to calculate the alveolar-arterial (A-A) oxygen gradient. All determinations were required to check in duplicate. The techniques used are detailed elsewhere (6). The pO_2 of mixed venous blood was obtained by plotting oxygen content (Van Slyke-Neill) on a standard oxyhemoglobin dissociation curve using the corresponding pH determined by the MacInnes-Belcher glass electrode. The oxygen and carbon dioxide tensions of arterial blood were determined directly (7), and the latter was also estimated from the line charts of Van Slyke and Sendroy, using the CO_2 content and the pH of the whole blood. Systolic and diastolic pressures were calculated as averages of two complete respiratory cycles; the mean pressure was obtained by planimetric integration of the area included within these boundaries. Variations were considered significant only if they exceeded limits previously defined in this laboratory (8–10) and include deviations greater than ± 5 mm. Hg for pulmonary artery mean pressure and -12 to $+18$ mm. Hg for systemic artery mean pressure. Similarly under the conditions of these experiments, any cardiac output change exceeding 9 per cent of control was considered significant.

The inspired gas mixtures used in these studies included 100 per cent oxygen and mixtures of 33, 25, 21, 18, 16, 14, and 12 per cent oxygen in nitrogen, administered through demand-type valves or from an anesthesia bag. At least two experiments, each at a different level of arterial oxygen saturation, were completed for each subject. In the following pages, "low oxygen" refers to inspired gas mixtures containing less than 21 per cent oxygen; conversely, "high oxygen" mixtures contain more than 21 per cent oxygen. The specific concentrations used are indicated in the tables.

Criteria used for defining the "steady state"

Three criteria were used as guides to stability of the respiration and circulation (the "steady state") as successive levels of oxygenation. These were *1*) emotional stability, *2*) constancy of oxygen consumption, and *3*) constancy of the respiratory exchange ratio (RQ). Significant deviations in any of these three criteria served to exclude a given subject from this study.

It is known that clinical manifestations of sympathetic overactivity are at best a crude guide to emotional disturbance. However, in response to emotional stimulation, oxygen consumption nearly always increases (11); in absence of such stimulation, oxygen consumption remains remarkably constant from day to day when determined under comparable conditions (12, 13). Neither acute anoxia *per se* nor the hyperventilation induced by it (14–16) causes increase of oxygen consumption. Because of these considerations and in keeping with a large body of data previously gathered in this laboratory during cardiac catheterization (8), any change in oxygen consumption which exceeded $+11$ per cent eliminated the experiment from inclusion in this study.

If the oxygen consumption is constant (within the limits defined above) at the two levels of oxygenation, an elevation in respiratory gas exchange ratio (R.Q.) becomes a reflection of respiratory rather than metabolic carbon dioxide liberation (unstable respiratory state). A review of a series of patients previously studied under comparable conditions in this laboratory (8) indicated that all of 20 patients had R.Q. values less than 1.0 during repeated determinations, and that 17 of the 20 deviated from the initial value by less than 0.13 during the second determination performed within one hour. In selecting the group to be presented below, any patient with pulmonary disease with any R.Q. greater than 1.0, or with a second R.Q. which differed from the first by more than 0.11, was excluded.

RESULTS

These data were derived from 45 complete studies on 35 subjects with pulmonary disease. The clinical diagnoses, physical findings, procedures and data concerning the steady state are indicated in Table I.

A. Ventilation

With three exceptions all of these subjects, while breathing ambient air, had a higher than normal respiratory minute volume (Table I). This observation is in accord with similar respiratory studies previously done on the same patients in this laboratory. In the experiments employing high oxygen, there was no significant change in ventilation. However, during low oxygen breathing, the volume of ventilation usually exceeded control levels.

B. Carbon dioxide tension in arterial blood (Table I)

Seventeen of 35 patients, while breathing ambient air, had normal (39.4 ± 2.8) arterial pCO_2. Ten had low pCO_2 values, probably secondary to their chronic hyperventilation. Eight, however, had elevated arterial pCO_2, the two highest values occurring in two subjects with emphysema and cor pulmonale (Cases 32 and 33). Following exposure to low oxygen, the arterial pCO_2 was usually lower than on ambient air. The arterial pCO_2 while the subjects were breathing a high oxygen mixture showed no consistent direction of change.

C. Heart rate

Resting heart rates ranged from 48 to 137. Exposure to high oxygen usually caused slowing or

TABLE I

Physical characteristics, clinical diagnoses, and orienting data in 35 patients studied at successive levels of oxygenation

Case	Sex	Age	BSA m^2	Diagnosis	Concentration inspired oxygen *volumes %*	Ventilation *lit./min./m²*	R.Q.	Oxygen consumption *cc./min./m²*	Arterial pCO_2 *mm. Hg*
(a) Normal and low oxygen									
1. G. W.	f	38	1.67	Bronchiectasis; interventricular septal defect	21	6.6	.87	113	40
					10	8.3	.82	124	31
2. L. K.	m	29	1.80	Diffuse pulmonary infiltration, cause unknown	21	5.1	.86	174	34
					12	7.2	.97	177	29
3. M. Mc	f	22	1.42	Diffuse pulmonary fibrosis, scleroderma	21.5	5.1	.85	133	42
					14	4.8	.85	132	37
4. J. L.	m	49	1.90	Silicosis; chr. pulm. emphysema	21	3.8	.87	134	36
					12	5.2	.91	148	36
5. D. P.	m	36	1.75	Bronchiectasis; diffuse pulmonary infiltration	21.5	3.5	.73	127	41
					18.5	3.9	.79	135	38
6. J. R.	m	40	1.38	Acute hematogenous tuberculosis	21	4.4	.83	112	40
					16	4.2	.94	102	37
					21.5	4.4	.86	124	37
					16	5.2	.84	128	36
7. T. Mc	f	30	1.59	Chr. pulm. tuberculosis	21	4.8	.86	129	43
					16	5.6	.86	141	40
8. E. H.	m	17	1.47	Severe diffuse pulmonary granulomata, cause unknown	21	8.3	.86	197	40
					16	8.9	.83	199	34
					21.5	7.6	.95	172	32
					16	7.0	.96	170	31
9. S. A.	m	60	1.65	Bronchiectasis; chr. pulm. emphysema	21	6.4	.94	123	41
					16	5.8	.98	128	38
					14	5.6	.97	125	39
10. Q. G.	f	43	1.66	Severe diffuse pulmonary granulomata, Boeck's sarcoid	21.5	6.5	.76	146	39
					17	7.2	.85	141	37
					21	6.3	.88	157	39
					17	6.8	.94	145	35
11. C. D.	m	23	1.67	Chr. pulm. tuberculosis	21	4.8	.72	157	34
					16	5.2	.80	146	32
12. H. K.	f	25	1.53	Bronchiectasis; bilateral lobectomy	21	5.0	.79	146	34
					16	5.0	.84	135	34
13. E. C.	m	29	1.69	Chr. pulm. tuberculosis	21	5.6	.82	125	39
					16	5.4	.88	120	36
14. K. C.	f	31	1.73	Acute hematogenous tuberculosis	21	3.7	.82	112	38
					16	3.8	.90	104	40
15. W. H.	m	22	1.90	Diffuse pulmonary granulomata, Boeck's sarcoid	21	4.7	.78	169	35
					16	6.5	.89	167	34
16. F. A.	m	48	1.55	Chr. pulm. tuberculosis; acute hematogenous tuberculosis	21	7.4	.85	147	35
					16	7.4	.83	148	35
17. F. L.	m	23	1.85	Severe diffuse pulmonary fibrosis, beryllium exposure	21	5.8	.78	140	37
					16	6.9	.88	131	35
18. R. S.	m	25	1.85	Moderate diffuse pulmonary granulomata, Boeck's sarcoid	21	4.3	.83	149	39
					16	4.7	.92	146	36
19. N. I.	m	31	1.35	Moderate diffuse pulmonary granulomata, Boeck's sarcoid	21	4.4	.82	121	34
					16	5.4	.91	128	35
20. C. L.	f	21	1.74	Moderate diffuse pulmonary granulomata, Boeck's sarcoid	21	7.0	.81	166	45
					16	7.9	.82	168	43

TABLE I.—*Continued*

Case	Sex	Age	BSA m^2	Diagnosis	Concentration inspired oxygen *volumes %*	Ventilation *lit./min./m²*	R.Q.	Oxygen consumption *cc./min./m²*	Arterial pCO_2 *mm. Hg*
(a) Normal and low oxygen—*Continued*									
21. J. R.	m	23	1.57	Acute hematogenous tuberculosis; 11 days after start Rx with streptomycin	21.5 16	5.5 5.7	.79 .86	147 140	40 36
(b) Normal, low and high oxygen									
22. B. B.	m	52	1.54	Bronchial asthma; chr. pulm. emphysema; cor pulmonale, not in failure	21 16 21 25	6.6 7.2 7.3 6.7	.84 .90 1.0 .90	137 151 150 146	47 42 41 39
23. A. P.	m	49	1.55	Chr. pulm. emphysema; cor pulmonale, in failure	21.5 16 21 30	5.8 6.5 5.4 5.9	.88 .97 .84 .93	141 144 127 128	45 46 42 42
24. D. Mc	m	62	1.61	Carcinoma of bronchus, RML; chr. pulm. emphysema	21 16 21 25	5.7 6.5 5.7 6.1	.80 .88 .80 .83	149 143 149 138	39 39 39 42
25. P. B.	f	42	1.56	Severe diffuse pulmonary granulomata, Boeck's sarcoid	12.5 17.4 21 25	7.4 8.0 7.4 8.3	.92 .96 .92 .98	156 157 156 164	40 36 40 40
26. W. B.	m	46	1.61	Bronchial asthma; chr. pulm. emphysema; cor pulmonale, not in failure	21.5 16 21 30	5.8 6.0 5.8 5.4	.85 .87 .85 .83	138 138 138 135	38 36 40 43
27. P. B.	m	36	1.49	Severe diffuse pulmonary granulomata, Boeck's sarcoid	21 17.4 21 25	8.8 8.8 8.8 8.8	.77 .83 .77 .82	203 184 205 187	35 32 37 36
28. M. L.	f	29	1.32	Severe diffuse pulmonary infiltration, cause unknown	21 17 21 25	7.3 7.3 6.4 6.5	.77 .84 .70 .80	147 141 153 141	29 28 37 39
(c) Normal and high oxygen									
29. J. P.	m	43	1.66	Bronchiectasis; cor pulmonale, in failure	21 100	6.9 —	.75 —	182 206	42 —
30. G. Na	f	52	1.87	Bronchial asthma; chr. pulm. emphysema	21.5 25	5.9 6.6	.72 .74	150 140	52 54
31. P. L.	m	65	1.60	Bronchial asthma; chr. pulm. emphysema	21.5 25 33	6.8 6.7 6.8	.72 .83 .78	141 131 138	30 35 45
32. A. Y.	m	38	1.47	Bronchial asthma; chr. pulm. emphysema; cor pulmonale in congestive failure	21 25	4.8 4.9	.79 .74	157 166	62 58
33. M. C.	m	55	1.54	Bronchial asthma; pulmonary fibrosis; chr. pulm. emphysema; cor pulmonale after treatment	21 25	6.2 6.1	.73 .70	168 161	58 55
34. E. M.	f	60	1.73	Severe diffuse pulmonary infiltration, cause unknown	21 100	5.8 —	.72 —	127 121	39 —
35. M. H.	f	64	1.39	Severe diffuse pulmonary infiltration, cause unknown; cor pulmonale, not in failure	21 30	10.0 9.3	.82 .80	144 140	43 49

TABLE II

The influence of the level of oxygenation upon the circulation in 35 patients with pulmonary disease

Case	Concentration inspired oxygen *volumes %*	Alveolar pO_2 *mm. Hg*	Arterial pO_2 *mm. Hg*	MVB pO_2 *mm. Hg*	Arteriovenous oxygen diff.* *volumes %*	Cardiac index *lit./min./m² BSA*	Heart rate *beats/min.*	Pulmonary artery pressure *mm. Hg*		Brachial artery pressure *mm. Hg*	
								s/d	*m*	*s/d*	*m*
(a) Normal and low oxygen											
1	21	97	73	44	2.1	6.20	100	25/10	18	131/76	99
	10	45	35	27	2.1	6.88	102	25/7	16	108/65	86
2	21	116	100	38	4.6	3.78	85	28/11	18	145/94	114
	12	62	46	31	3.4	5.19	98	32/12	20	123/79	95
3	21.5	100	94	37	3.7	3.60	79	32/13	20	95/57	74
	14	57	29	22	1.8	7.33	98	41/18	27	110/69	84
4	21	111	75	35	5.5	2.43	75	32/11	21	120/68	89
	12	50	36	27	4.0	3.71	88	54/20	36	135/75	103
5	21.5	103	75	33	5.3	2.39	48	36/12	19	136/83	106
	18.5	89	68	31	5.1	2.65	55	41/12	24	134/80	106
6	21	108	102	34	4.2	2.65	85	12/4	9	104/71	84
	16	78	70	33	3.5	2.96	88	17/7	12	111/75	90
	21.5†	108	84	30	4.9	2.38	68				
	16†	69	52	27	4.5	2.67	74				
7	21	107	77	40	3.6	3.68	78	24/6	15	116/64	86
	16	77	63	32	3.7	3.80	75	22/6	14	110/61	81
8	21	107	74	36	4.1	4.80	107	34/18	26	98/64	77
	16	72	43	28	4.1	4.88	115	41/23	31	82/55	67
	21.5†	113	94	38	3.9	6.95	90	39/17	27	132/83	106
	16†	73	56	32	4.1	6.56	100	40/23	31	124/80	98
9	21	107	66	34	4.5	2.67	86	25/12	17	139/77	102
	16	71	47	31	4.4	2.85	88	31/13	20	127/73	92
	14	58	38	30	4.0	3.05	88	35/15	24	127/80	102
10	21.5	107	56	27	4.3	3.39	94	55/27	37		
	17	88	36	25	3.3	4.21	94	67/28	43	115/67	87
	21†	99	71	30	3.7	4.23	106	46/21	32	109/68	85
	17†	78	53	27	3.2	4.52	103	56/23	42	113/67	85
11	21	114	78	37	5.1	3.09	80	18/8	12	122/77	94
	16	74	64	34	4.7	3.10	80	19/7	13	129/83	98
12	21	107	88	36	3.5	3.90	72	26/9	16	126/74	99
	16	72	60	32	3.3	4.10	82	31/10	20	130/77	100
13	21	105	69	35	4.7	2.64	80	23/10	16	114/77	94
	16	64	52	32	4.3	2.80	80	25/11	18	115/72	92
14	21	102	96	38	4.5	2.49	60	23/7	13	128/85	104
	16	72	66	36	3.9	2.68	74	30/9	18	121/82	101
15	21	110	92	40	3.5	4.83	74	17/9	13	124/82	99
	16	75	75	36	3.0	5.58	78	19/10	14	116/80	96
16	21	109	84	32	4.8	3.04	80	24/8	15	118/77	95
	16	67	54	30	5.1	2.90	80	28/10	18	108/71	89
17	21	109	86	36	4.9	2.85	80	30/14	21	116/85	101
	16	71	47	30	4.9	2.68	79	37/17	25	115/83	97
18	21	106	88	42	3.8	3.92	98	20/8	13	151/92	118
	16	78	74	36	4.1	3.82	96	30/10	20	141/88	110
19	21	104	98	30	4.1	2.96	97	22/8	15	111/69	87
	16	83	82	28	3.8	3.37	105	22/9	16	113/67	86
20	21	110	75	34	4.4	3.82	91	32/10	21	127/73	91
	16	75	47	28	4.5	3.73	93	32/14	24	120/76	92

TABLE II.—*Continued*

Case	Concentration inspired oxygen *volumes %*	Alveolar pO_2 *mm. Hg*	Arterial pO_2 *mm. Hg*	MVB pO_2 *mm. Hg*	Arteriovenous oxygen diff.* *volumes %*	Cardiac index *lit./min./m² BSA*	Heart rate *beats/min.*	Pulmonary artery pressure *mm. Hg*		Brachial artery pressure *mm. Hg*	
								s/d	*m*	*s/d*	*m*
(a) Normal and low oxygen—*Continued*											
21	21.5	105	88	34	3.7	3.97	102	17/7	13	124/76	93
	16	69	66	30	3.1	4.50	102	14/7	11	127/77	93
(b) Normal, low, and high oxygen											
22	21	94	62	36	4.1	3.33	90	26/11	15	109/62	80
	16	69	55	32	4.1	3.70	103	49/20	32	119/74	92
	21	110	60	37	4.6	3.43	93	39/18	27	142/83	104
	25	141	71	38	4.8	3.20	93	31/17	22	119/76	94
23	21.5	101	60	22	3.9	3.62	95	35/17	24	102/63	79
	16	71	36	18	4.0	3.59	94	49/20	32	119/74	92
	21†	104	61	29	4.7	2.71	68	36/9	19	121/61	85
	30†	189	100	33	4.7	2.71	62	39/12	22	137/67	94
24	21	101	78	34	4.2	3.55	83	29/8	17	143/70	99
	16	72	63	32	3.7	3.86	88	35/9	19	142/72	100
	21	101	78	34	4.2	3.55	83	29/8	17	143/70	99
	25	129	87	36	5.1	2.71	83	31/7	17	153/74	104
25	21.5	118	84	28	5.1	3.07	87	59/27	37	170/105	131
	17.4	94	57	26	4.2	3.74	90	60/23	34	169/109	133
	21	112	84	28	5.1	3.07	90	59/27	37	183/112	135
	25	139	95	30	4.9	3.35	90	52/22	34	190/117	143
26	21.5	107	67	32	5.8	2.39	56	28/7	16	136/79	106
	16	66	40	29	4.8	2.88	66	32/11	20	128/77	101
	21	107	67	33	5.8	2.39	56	28/7	16	136/79	106
	30	167	110	40	5.6	2.41	60	30/9	17	141/80	102
27	21	103	63	19	5.9	3.45	100	50/23	34	91/65	77
	17.4	83	46	16	5.3	3.46	90	59/27	39	91/63	74
	21	103	63	20	5.9	3.45	100	50/23	34	91/65	77
	25	133	81	24	6.0	3.12	100	65/31	42	110/79	92
28	21	114	65	33	4.2	3.12	80	34/16	23	108/71	89
	17	82	40	31	3.2	3.94	80	48/23	33	105/71	87
	21	114	65	34	4.3	3.15	73	36/18	26	97/67	81
	25	142	77	37	4.0	3.14	76	34/17	24	99/66	81
(c) Normal and high oxygen											
29	21	95	54	37	3.5	5.20	137	42/19	31	129/88	108
	100	—	104	76	4.4	4.68	129	40/17	28	131/88	110
30	21.5	88	48	30	4.5	3.62	75	42/18	28	118/49	75
	25	109	60	31	4.7	3.17	78	42/18	27	124/50	76
31	21.5	116	64	34	4.0	3.53	76	22/7	12	140/82	108
	25	139	75	34	3.9	3.35	74	24/8	15	146/88	114
	33	190	83	34	3.9	3.52	73	20/6	11	138/82	105
32	21	73	46	33	3.4	4.63	106	43/21	30	112/72	84
	25	102	75	38	3.4	4.89	114	51/24	38	139/86	105
33	21	72	33	21	5.6	3.00	115	74/32	46	121/84	99
	25	102	50	28	5.6	2.88	108	77/36	51	114/78	92
34	21	98	82	34	3.9	3.24	91	58/20	34	121/69	91
	100	—	100	111	4.3	2.83	85	—	31	127/69	94
35	21	105	45	26	5.6	2.57	88	67/26	41	120/59	84
	30	194	84	33	5.7	2.46	88	60/28	40	125/62	89

* Using mixed venous blood obtained from the pulmonary artery.
† These observations were made on a second day.

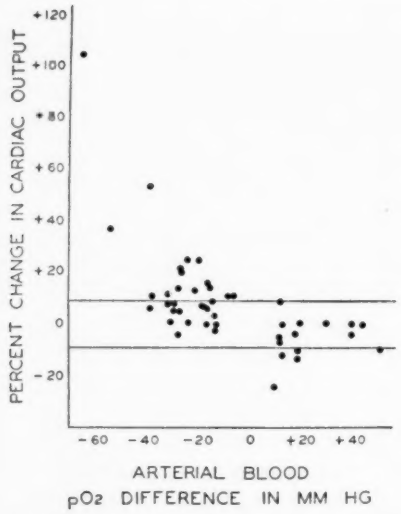

FIG. 1A. RELATION BETWEEN CHANGES IN ARTERIAL OXYGEN TENSION AND CARDIAC OUTPUT

The cardiac output change is expressed in per cent of change from the initial measurement. A change of 9 per cent (above and below the two solid lines) is significant.

Note that during anoxia, cardiac output remains unchanged or rises and that increased oxygenation causes no change or a decrease in cardiac output.

insignificant change in heart rate (Table II). A persistent tachycardia was observed in seven of 28 patients during exposure to low oxygen.

D. Cardiac output

There was considerable variation in the level of the initial cardiac index in these experiments (28 normal [3.09 ± 0.5 lit./min./m² B.S.], five low and 12 greater than normal). All 12 with high resting cardiac output had advanced pulmonary disease. Three (Cases 23, 29, 32) were in right heart failure; one (Case 1) had an associated interventricular septal defect. The effects of variation in level of oxygenation on cardiac output are illustrated in Figures 1 and 2. It is apparent that during exposure to low oxygen, the cardiac output either failed to change significantly (less than ± 9 per cent) or increased. There was a significant increase in 14 of the 32 cases. In no instance was there a significant decrease. In 10 additional cases there were small increases, but less than the error of the method. The net experience suggests at least a tendency toward increased cardiac output during moderate anoxia in this series of cases.

On the other hand when we applied the Fick principle to a group of patients with similar clinical diagnoses, but which had been rejected from this study because, according to our criteria, they had failed to achieve a "steady state," the calculated cardiac outputs were found to vary greatly, often with values considerably below control. Since values for cardiac output so obtained are meaningless, we have not tabulated them.

Tables II and III further demonstrate that exposure to high oxygen in the steady state either effected no significant change or a decrease in cardiac output. The degree of change in cardiac output could not be quantitatively correlated with the concentration of oxygen in the inspired gas mixture. This is to be anticipated in the presence of chronic pulmonary disease, particularly in patients with alteration of the alveolar-capillary membrane (for instance Case 3), where decrease in inspired oxygen concentration will cause a greater decrease in arterial oxygen saturation than in normal subjects. The greatest changes in cardiac output were associated with marked changes in

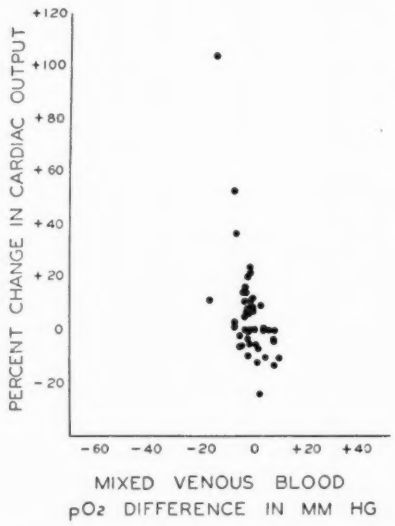

FIG. 1B. RELATION BETWEEN CHANGES IN MIXED VENOUS BLOOD OXYGEN TENSION AND CARDIAC OUTPUT

Note that mixed venous blood oxygen tension is not related to cardiac output.

arterial oxygen tension (Figure 1a). There was no significant relation between mixed venous oxygen tensions and cardiac output (Figure 1b).

E. Pulmonary artery pressure (Table II)

Following increase in the concentration of inspired oxygen, there was no significant change in mean pulmonary artery pressure, except in two subjects (cases 27 and 32), where the mean pulmonary artery pressure rose by 8 mm. Hg.

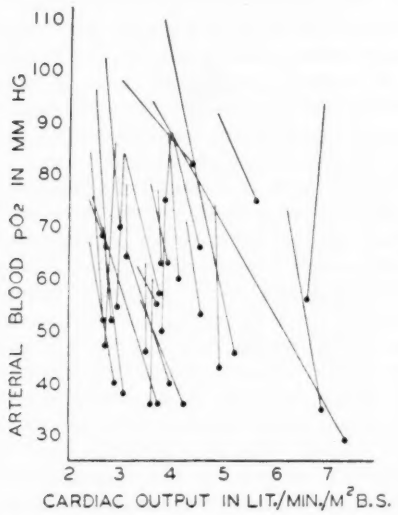

FIG. 2A. THE RESPONSE OF CARDIAC OUTPUT TO ACUTE ANOXIA IN INDIVIDUAL PATIENTS

The upper end of each line indicates the arterial oxygen tension on 21 per cent oxygen; the lower knobbed end indicates the arterial oxygen tension during anoxia.

Note that the slope of the lines indicates that the cardiac output increased or did not change significantly during anoxia.

During low oxygen, mean pulmonary artery pressure rose more than 5 mm. Hg in 10 of 38 experiments. Of the remaining 28 observations, 11 had increases in pulmonary artery pressure, during anoxia, of 3 to 5 mm. Hg. Not all subjects with increased pressures during anoxia had increased cardiac outputs. However, the subjects (Cases 4 and 22) with the largest increases in pulmonary artery pressure had significant simultaneous increases in cardiac output.

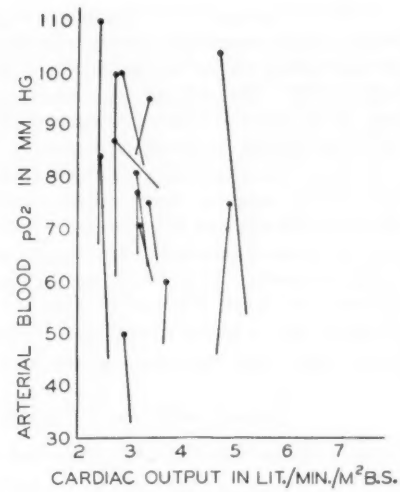

FIG. 2B. THE RESPONSE OF CARDIAC OUTPUT TO INCREASE IN ARTERIAL OXYGEN TENSION IN INDIVIDUAL PATIENTS

The lower end of each line indicates the arterial oxygen tension on 21 per cent oxygen; the upper knobbed end indicates the arterial oxygen tension at the higher level of oxygenation.

Note that at these higher levels of arterial oxygen tension there was either no significant change or a fall in cardiac output.

F. Brachial artery pressure

The changes in brachial artery pressure following high and low oxygen showed no consistent trend. Only one subject (Case 32) underwent a large change in mean pressure (+ 21 per cent) while breathing high oxygen.

DISCUSSION

The importance of achieving the "steady state" when one determines cardiac output by the Fick principle [4] has been emphasized in this presenta-

[4] The Fick principle states that the quantity of any substance taken up by blood perfusing an organ is equal to the amount of that substance in the volume of blood leaving the organ minus the amount contained in the volume of blood entering the organ. For the instantaneous uptake of oxygen by blood in the lungs

$$\dot{V}_{O_2} = \frac{dV_{O_2}}{dt} = [Ca_{O_2}(t) - Cv_{O_2}(t)]Q(t), \quad \text{Equation 1}$$

where Q = instantaneous blood flow *to* lungs = instantaneous blood flow *from* lungs at time (t) and Ca_{O_2}, Cv_{O_2} = instantaneous concentrations of oxygen in arterial

tion. Failure to recognize the importance of this factor may explain some of the discrepancies in previous observations on the effects of anoxia upon the circulation. The difficulty in achieving a "steady state," even in normal subjects exposed to moderate anoxia, is clearly indicated by the data of Rahn and Otis (15). In their studies, normal trained subjects were acutely elevated to simulated altitude, and the time was noted for restoration of heart rate, R.Q., and oxygen consumption to normal. They found that the higher the altitude, the longer the time required for re-equilibration, and at 10 per cent oxygen (roughly equivalent to 18,000 feet) an hour or more was required for these measurements to return to control levels. It is apparent that if our patients had been exposed to these levels of anoxia, a steady state would have been difficult or impossible to approach during the time (15 minutes or more) allotted for equilibration. This is emphasized by the large number of patients who were excluded from this presentation because of their inability to reach a "steady state" even with mild depression of inspired oxygen content.

In our subjects presented above, despite the presence of advanced lung disease, it was possible to approximate a "steady state" by careful selection of the inspired oxygen mixture so as to avoid extreme variations in arterial oxygen saturation. Under these conditions, no significant fall in cardiac output was ever observed during anoxia. This finding led us to re-evaluate the observations on cardiac output previously recorded from this laboratory (3) incident to a study of changes in pulmonary artery pressure in five normal subjects who were exposed to low oxygen mixtures (10 per cent) for brief periods (about 10 minutes). The average cardiac output of the five subjects was reported to have decreased from 5.74 liters to 5.20 liters. A review of the original data from which these results were calculated indicated that these subjects were not in a "steady state" (high initial R.Q., marked variations in successive R.Q. values, and unusually low oxygen intake at the low level of oxygenation). Similar considerations apply to the fall in cardiac output during acute anoxia recently reported from another laboratory (4). Such evidence of an unsteady state invalidates the use of the Fick principle for calculation of cardiac output.

The need for a "steady state" prior to the application of the Fick principle obviously extends to conditions other than acute anoxia, *e.g.*, exercise. Bock, Dill, and their associates (17) stressed the difficulties in reaching a "steady state" during exercise, and urged caution in the interpretation of measurements so made. The evaluation of measurements of cardiac output after exercising patients with heart disease for three minutes is consequently difficult (18).

The other recorded changes in the circulation in man are generally in accord with previous data

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and mixed venous blood respectively, at time (t), from which,

$$Q(t) = \frac{\dot{V}_{O_2}}{Ca_{O_2} - Cv_{O_2}} \qquad \text{Equation 2}$$

and generalizing, the mean flow ($\bar{Q}$) during time (t), becomes

$$\bar{Q} = \frac{1}{T}\int_0^T \frac{\dot{V}_{O_2}}{Ca_{O_2} - Cv_{O_2}}\,dt. \qquad \text{Equation 3}$$

When the quantities V_{O_2}, $Ca_{O_2} - Cv_{O_2}$ remain constant (or do not significantly differ from the mean), then,

$$\bar{Q} = \frac{\bar{\dot{V}}_{O_2}}{\overline{Ca_{O_2} - Cv_{O_2}}}. \qquad \text{Equation 4}$$

In this equation $\bar{Q}$, $\bar{\dot{V}}_{O_2}$, and $\overline{Ca_{O_2} - Cv_{O_2}}$ represent mean values during the time t. This is the form of the Fick principle generally used for calculation of cardiac output. Implicit to its application are the concepts that oxygen is neither secreted nor stored in the lungs.

For substitution in Equation 4, it is assumed that the oxygen uptake, as measured from collection and analysis of expired gas, is equal to the oxygen taken up by the blood in its passage through the lungs; which in turn is equal to the oxygen consumed by the tissues. This relationship does not obtain during the period immediately following the change-over from ambient air to the anoxic mixture. During this period of adjustment to the acute anoxia, the quantity of oxygen in the blood phase and the gas phase is greater than will exist when equilibrium is achieved. Consequently tissue need for oxygen will be met not only from the inspired low oxygen mixture, but also from the surplus oxygen retained in the circulating blood and lungs from the previous level of oxygenation. The oxygen consumption as measured from the spirometer ("apparent oxygen consumption") will thus be lower than both the actual tissue oxygen consumption and the oxygen taken up by the blood traversing the lungs. Cardiac output calculated from this "apparent oxygen consumption" will be lower than the true cardiac output.

Another difficulty in the unsteady state is to relate the measured oxygen uptake to the blood samples responsible for its uptake. Simultaneous and prolonged collection periods for blood and gas do not completely solve this problem, since the proper time relationship is unpredictable.

TABLE III

Summary of changes in circulation and respiration at two levels of oxygenation

Case*	Final concentration inspired oxygen† %	Ventilation‡ %	Oxygen consumption‡ %	R.Q.‡	pO_2 arterial blood‡ *mm. Hg*	pO_2 MVB‡ *mm. Hg*	Heart rate‡ *beats/min.*	Cardiac index‡ %	Pulmonary artery pressure‡ *mean mm. Hg*	Brachial artery pressure‡ *mean mm. Hg*
(a) From normal to low oxygen										
1	10	+25.8	+11	−.05	−38	−17	+ 2	+ 11	− 2	−13
2	12	+41.2	+ 1.5	+.11	−54	− 7	+13	+ 37	+ 2	− 9
3	14	− 5.9	0	0	−65	−15	+19	+104	+ 7	+10
4	12	+36.9	+11	+.04	−39	− 8	+13	+ 53	+15	+14
5	18.5	+11.4	+ 6.8	+.06	− 9	− 2	+ 7	+ 11	0	0
6	16	− 4.2	− 8.6	+.11	−32	− 1	+ 3	+ 12	+ 3	+ 6
	16	+27	+ 0.5	−.02	−32	− 3	+ 6	+ 8		
7	16	+ 9.2	+ 9	0	−14	− 8	− 3	+ 3	− 1	− 5
8	16	+ 8.3	+ 3	−.03	−31	− 8	+ 8	+ 1	+ 5	− 9
	16	− 7.9	0	+.01	−28	− 6	+10	− 6	+ 4	− 8
9	16	− 8.6	+ 4.4	+.04	−19	− 3	+ 3	+ 7	+ 3	− 4
	14	−12.4	+ 1.5	+.03	−28	− 4	+ 6	+ 14	+ 7	+ 3
10	17	+10.7	− 3.4	+.09	−20	− 2	0	+ 24	+ 6	
	17	+ 7.9	− 5.1	+.06	−18	− 3	− 3	+ 7	+10	0
11	16	+ 8.3	− 7.5	+.08	−14	− 3	0	0	+ 1	+ 4
12	16	0	− 6.2	+.05	−28	− 4	+10	+ 5	+ 4	+ 1
13	16	− 3.6	− 4.0	+.06	−17	− 3	0	+ 6	+ 2	− 2
14	16	+ 2.7	− 7.2	+.08	−30	− 2	+14	+ 8	+ 5	− 3
15	16	+40.0	0	+.11	−17	− 4	+ 4	+ 16	+ 1	− 3
16	16	0	0	−.02	−30	− 2	0	− 5	+ 3	− 6
17	16	+19.0	− 6.4	+.10	−39	− 6	− 1	− 6	+ 4	− 4
18	16	+ 9.3	0	+.09	−14	− 6	− 2	− 3	+ 7	− 8
19	16	+22.5	+ 5.8	+.09	−16	− 2	+ 8	+ 14	+ 1	− 1
20	16	+12.8	0	+.01	−28	− 6	+ 2	− 2	+ 3	+ 1
21	16	+ 3.6	− 4.8	+.07	−22	− 4	0	+ 13	− 2	0

from this laboratory (3), and, in animals, from other laboratories (19). Motley and his associates (3) found that pulmonary artery pressure uniformly increases in normal subjects acutely exposed to 10 per cent oxygen. Approximately one-third of our patients with pulmonary disease manifested a similar response to anoxia. However, the failure of 28 of the 38 to have a significant increase in pulmonary artery pressure at the end of 15 to 20 minutes of anoxia deserves comment. At least three other factors may be considered in evaluating this finding: *1*) The observed tendency of the elevated pulmonary artery pressures to fall towards initial levels in many of these patients as breathing at the low level of oxygenation is continued; *2*) the difference which may exist between the response of the chronically anoxic (acclimatized) and normal subject to acute anoxia; and *3*) the significantly higher pO_2 alveolar level attained in these studies when compared to the previous studies in normal animals and man (3, 19).

Whether a single mechanism is involved in the

TABLE III—*Continued*

Case*	Final concentration inspired oxygen† %	Ventilation‡ %	Oxygen consumption‡ %	R.Q.‡	pO_2 arterial blood‡ *mm. Hg*	pO_2 MVB‡ *mm. Hg*	Heart rate‡ *beats/min.*	Cardiac index‡ %	Pulmonary artery pressure‡ *mean mm. Hg*	Brachial artery pressure‡ *mean mm. Hg*
				(b) From normal to low and to high oxygen						
22	16	+ 9.1	+11	+.06	− 7	− 4	+13	+ 11	+17	+12
	25	− 8	− 2	−.11	+11	+ 1	0	− 7	− 5	−10
23	16	+ 8.3	+ 2	+.09	−24	− 4	− 1	0	+ 8	+13
	30	+ 9.3	0	+.09	+39	+ 4	− 6	0	+ 3	+ 9
24	16	−14.0	+ 0.5	+.08	−15	− 2	+ 5	+ 9	+ 2	
	25	+ 7	+ 7.5	+.03	+ 9	+ 2	0	− 24	0	+ 5
25	17.4	+ 8.1	0	+.04	−27	− 2	+ 3	+ 22	− 3	+ 2
	25	+12.1	+ 5.8	+.06	+11	+ 2	0	+ 9	− 3	+ 8
26	16	+ 3.5	0	+.02	−27	− 3	+10	+ 20	+ 4	− 5
	30	− 6.9	− 2.2	−.02	+43	+ 7	+ 4	0	+ 1	− 4
27	17.4	0	− 9.3	+.06	−17	− 3	−10	0	+ 5	− 3
	25	0	− 8.8	+.05	+18	+ 4	0	− 10	+ 8	+15
28	17	0	− 4.1	+.07	−25	− 2	0	+ 25	+10	− 2
	25	+ 1.6	− 7.9	+.10	+12	+ 3	+ 3	0	− 2	0
				(c) From normal to high oxygen						
29	100		+11		+50	+ 9	− 8	− 10	− 3	+ 2
30	25	+12	− 6	−.01	+12	+ 1	+ 3	− 12	+ 3	+11
31	25	0	− 8	+.11	+11	0	− 2	− 5	− 3	+ 6
	33	0	− 3	+.06	+19	0	− 3	0	− 1	− 3
32	25	+ 1	+ 5	−.05	+29	+ 5	+ 8	0	+ 8	+21
33	25	− 1	− 4	−.03	+17	+ 7	+ 3	− 4	+ 5	− 7
34	100		− 4		+18	+ 7	− 6	− 13	− 3	+ 3
35	30	− 7.0	− 2.7	−.02	+39	+ 7	0	− 4	− 1	+ 5

* As in Tables I and II.
† Second level of oxygenation. Initial level in all instances was ambient or compressed air.
‡ As compared to study with ambient or compressed air.

cases in whom the pulmonary artery pressure increased during anoxia is not clear. Indeed, our data do not provide any information concerning the contribution of the pulmonary veins or left auricle to the elevation in the pulmonary arterial pressure. However, in this group of patients with chronic pulmonary disease, an increase of blood flow in a pathologically restricted vascular bed may cause a rise in pulmonary arterial pressure. This factor could be invoked in seven of the 10 subjects in whom an increase in pulmonary arterial pressure was observed following low oxygen breathing.

Changes in cardiac output greater than 15 per cent at the lower level of oxygenation occurred in nine of the 47 determinations. Such marked changes are of particular importance in the method which Riley, Cournand and Donald (6) have recently described for estimating the oxygen-diffusing capacity and the ventilation-perfusion relationships of the lung. Our data suggest that the assumption of a relatively constant cardiac output in subjects exposed to two levels of inspired oxygen, an assumption which is essential to their method, is most apt to be valid if extreme variation in arterial oxygen tension is avoided by careful selection of inspired oxygen mixtures and the use of an oximeter. However, even with this precau-

tion, a significant, but at the present time unpredictable, increase in cardiac output may occur in some patients with pulmonary disease.

SUMMARY

1. The circulatory responses of 35 patients with pulmonary and cardiopulmonary disease to selected levels of oxygenation (higher and lower than room air) were investigated.

2. Particular care was exerted to arrive at a "steady state" of the respiration and circulation. The criteria for the "steady state" are defined; the relation of the "steady state" to the applicability of the Fick principle for cardiac output measurement is discussed.

3. After the "steady state" was achieved in the patients with chronic pulmonary disease exposed to moderate anoxia, cardiac output remained unchanged or increased. Conversely, an increase in concentration of inspired oxygen caused either no change or occasionally a slight fall in cardiac output. The largest increases in cardiac output during anoxia were associated with marked decreases in arterial oxygen pressure.

4. In response to anoxia, pulmonary artery pressure increased significantly (more than 5 mm. Hg) in a third of these patients with pulmonary disease and remained unchanged in the others.

ACKNOWLEDGMENT

The authors gratefully acknowledge the aid which they received from Professor Georges A. Deschamps in the preparation of the footnote.

REFERENCES

1. Grollman, A., The Cardiac Output of Man in Health and Disease. Charles C Thomas Co., Springfield, 1932.
2. Harrison, T. R., Wilson, C. P., Neighbors, D. W., and Pilcher, C., The regulation of the circulation. VII. The effects of anoxemia of mild degree on the cardiac output of unnarcotized dogs. Am. J. Physiol., 1927–28, **83**, 275.
3. Motley, H. L., Cournand, A., Werkö, L., Himmelstein, A., and Dresdale, D., The influence of short periods of induced acute anoxia upon pulmonary artery pressures in man. Am. J. Physiol., 1947, **150**, 315.
4. Westcott, R. N., Fowler, N. O., Scott, R. C., Hauenstein, V. D., and McGuire, J., Anoxia and human pulmonary vascular resistance. J. Clin. Invest., 1951, **30**, 957.
5. Lilienthal, J. L., Jr., Riley, R. L., Proemmel, D. D., and Franke, R. E., An experimental analysis in man of the oxygen pressure gradient from alveolar air to arterial blood during rest and exercise at sea level and at altitude. Am. J. Physiol., 1946, **147**, 1919.
6. Riley, R. L., Cournand, A., and Donald, K. W., Analysis of factors affecting partial pressures of oxygen and carbon dioxide in gas and blood of lungs; methods. J. Appl. Physiol., 1951, **4**, 102.
7. Riley, R. L., Proemmel, D. D., and Franke, R. E., A direct method for determination of oxygen and carbon dioxide tensions in blood. J. Biol. Chem., 1945, **161**, 621.
8. Harvey, R. M., Ferrer, M. I., Cathcart, R. T., Richards, D. W., Jr., and Cournand, A., Some effects of digoxin upon the heart and circulation in man. Digoxin in left ventricular failure. Am. J. Med., 1949, **7**, 439.
9. Ferrer, M. I., Harvey, R. M., Cathcart, R. T., Webster, C. A., Richards, D. W., Jr., and Cournand, A., Some effects of digoxin upon the heart and circulation in man. Digoxin in chronic cor pulmonale. Circulation, 1950, **1**, 161.
10. Cournand, A., The Fourth Walter Wile Hamburger Memorial Lecture, Institute of Medicine of Chicago. Some aspects of the pulmonary circulation in normal man and in chronic cardiopulmonary diseases. Circulation, 1950, **2**, 641.
11. Ziegler, L. H., and Levine, B. S., The influence of emotional reactions on basal metabolism. Am. J. M. Sc., 1925, **169**, 68.
12. Benedict, F. G., Degree of constancy in human basal metabolism. Am. J. Physiol., 1934–35, **110**, 521.
13. Du Bois, E. F., Basal Metabolism in Health and Disease. Lea & Febiger, Philadelphia, 1936, 3rd ed.
14. Grollman, A., Physiological variations of the cardiac output of man. VII. The effects of high altitude on cardiac output and its related functions: an account of experiments conducted on the summit of Pikes Peak, Colorado. Am. J. Physiol., 1930, **93**, 19.
15. Rahn, H., and Otis, A. B., Man's respiratory responses during and after acclimatization to high altitude. Am. J. Physiol., 1949, **157**, 445.
16. Houston, C. S., and Riley, R. L., Respiratory and circulatory changes during acclimatization to high altitude. Am. J. Physiol., 1947, **149**, 565.
17. Bock, A. V., Van Caulaert, C., Dill, D. B., Fölling, A., and Hurxthal, L. M., Studies in muscular activity. IV. The "steady state" and the respiratory quotient during work. J. Physiol., 1928, **66**, 162.
18. Gorlin, R., Sawyer, C. G., Haynes, F. W., Goodale, W. T., and Dexter, L., Effects of exercise on circulatory dynamics in mitral stenosis. III. Am. Heart J., 1951, **41**, 192.
19. v. Euler, U. S., and Liljestrand, G., Observations on the pulmonary arterial blood pressure in the cat. Acta physiol. Scandinav., 1946, **12**, 301.

THE REACTIVITY OF THE CAPILLARY BED OF THE NAILFOLD TO CIRCULATING EPINEPHRINE AND NOR-EPINEPHRINE IN PATIENTS WITH NORMAL BLOOD PRESSURE AND WITH ESSENTIAL HYPERTENSION

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(Submitted for publication January 24, 1952; accepted June 20, 1952)

Several studies have attempted to determine whether the vascular system of patients with essential hypertension is hyper-reactive to epinephrine. In these studies vascular reactivity was judged solely by the increase in arterial blood pressure following the parenteral administration of this pressor substance. Some investigators believed that this method indicated vascular hyper-reactivity to epinephrine in patients with essential hypertension; others concluded that the vascular reactivity remained within the normal range (1–7).

Epinephrine does not alter blood pressure by a single mechanism. Some vascular beds (skin and splanchnic area) are constricted, others (muscular) are dilated by the drug. The resultant change in the overall peripheral resistance depends upon which effect predominates. Epinephrine also acts directly upon the heart, and increases the cardiac output. Alteration of blood pressure following epinephrine administration is therefore a resultant of the changes of three separate mechanisms—vasoconstriction, vasodilatation, and cardiac output. A hyper-reactivity to epinephrine of those vascular beds that react by vasoconstriction may be masked if blood pressure changes are employed as the sole criteria of vascular reactivity.

An approach which would assay vasoconstrictor reactivity alone consists of direct observation of the changes produced in the caliber of the lumen of the minute vessels when pressor substances are injected. This approach entails several major disadvantages: *1*) the number of capillary beds accessible for examination in the intact individual is limited to those located peripherally; *2*) small variations in the diameter of the individual vessel, which could appear insignificant, might actually be significant, when multiplied by the tremendous number of vessels involved in a similar response; *3*) a vascular bed which is already partially or fully constricted would show little or no change in response to a pressor substance regardless of its intrinsic reactivity.

The last two difficulties are minimized by observing the narrowest contractile elements of the minute vascular bed. A slight constriction of these vessels markedly reduces the stream of red blood cells flowing distally and produces a readily visible effect, ischemia of the capillary bed. If the capillary bed is already partially or fully constricted, ischemia is noted and injection of the pressor substances is withheld.

It has recently been demonstrated that the precapillaries and metarterioles of the bulbar conjunctiva have fairly definite ranges of reactivity to topically applied epinephrine, and that in individuals with normal blood pressure the ranges are different from patients with essential hypertension. In patients with essential hypertension these minute vessels were found to be hyper-reactive to *topically* applied epinephrine. Only the vascular bed of the *bulbar conjunctiva* of individuals in the upright position was studied (8, 9).

The present study was undertaken to determine whether the reactivity of a minute vascular bed to *circulating* epinephrine and nor-epinephrine differed significantly in patients with normal blood pressure from patients with essential hypertension. The capillary bed of the nailfold of the finger was selected for observation.

METHOD

Ward patients with normal blood pressures and those with essential hypertension were selected for study. Patients with associated disease states that independently might have altered the peripheral vascular reactivity were usually excluded. This applied to subjects with an abnormal blood volume, cardiac output, or peripheral circulation, and to those receiving medications known to

induce vasomotor changes. The patients were recumbent for at least one-half hour, or until their blood pressures had stabilized. A needle with a three way stopcock was inserted into the antecubital vein of one arm. A tuberculin syringe was attached to this arrangement so that calibrated doses of pressor substances could be injected intravenously without the knowledge of the patient. The other arm was kept approximately 30 degrees from the side with the fingertips at the level of the sternum and steadied by a clamp applied to the lateral margins of the distal phalanx. Cedar oil was applied to the surface of the nailfold and the capillary microscope focused upon the terminal capillary loops which were illuminated by means of a slit lamp. The capillary bed was then examined until two adjacent capillary loops with arterial segments approximately 8 microns in diameter were found. These were selected for observation. Graded doses of one of the pressor substances, epinephrine [1] (E), levo-epinephrine [2] (E1), or levo nor-epinephrine [3] (N) were then injected intravenously in increments of 0.25 to 0.50 gamma approximately every five minutes until vasoconstriction, recognized by a definite ischemia of the arterial segments of these two loops, persisted for at least 15 seconds. The same dose was then reinjected five minutes later to determine the reproducibility of the effect. When the repeat injection occasionally failed to produce the same result, the injections were continued with progressively larger quantities until a reproducible effect was obtained. This quantity was recorded as the "threshold" epinephrine capillary reactivity.

The rate of injection was kept as uniform as possible at 0.4 cc. per second, the volume usually not exceeding 0.8 cc. The epinephrine and nor-epinephrine solutions were freshly prepared prior to injection and so constituted that 1 cc. always contained 2.5 gamma of the free base in isotonic saline.

Reflex vasodilatation was not employed. However, special attention was paid to the temperature and color of the extremities which, together with direct observations of the rate of blood flow in the capillaries, the degree of clumping of the red blood cells, and the circulation time, served as a satisfactory index of the adequacy of peripheral capillary flow. The circulation time was measured from the moment of injection of epinephrine into the antecubital vein to the initiation of ischemia of the capillary loops of the nailfold.

RESULTS

The anatomy of the capillary bed of the nailfold has been described previously (10). The terminal capillary loops were usually clearly visualized. The arterial segment of each capillary loop manifested independent and intermittent vasoconstriction, during which ischemia of the arterial segment occurred. This intermittent and independent vasoconstriction of the capillary bed is known as "vasomotion" (11–13). In patients with normal arterial blood pressure and in those with essential hypertension the *duration* of the constricted phase of this normal vasomotion process was brief, usually less than two seconds. The *frequency* of vasoconstriction varied markedly even in the same capillary loop. The majority of the capillary loops in patients with normal blood pressure remained patent with a fairly steady rate of blood flow and with only occasional vasoconstriction. In patients with essential hypertension, exposed to similar room temperatures and with comparably warm hands to the touch, there was usually an increase in the frequency of constriction of the arterial segments of the capillary loops. The interval between the constricted phases would occasionally be as brief as five seconds. A similar increased vasomotion has been described in the minute vascular bed of the bulbar conjunctiva of individuals with essential hypertension (9).

The intravenous injection of epinephrine and nor-epinephrine appeared to intensify the normal vasomotion process. As the quantity of epinephrine or nor-epinephrine injected was increased, the duration and the frequency of the constriction of the arterial segment of each capillary loop increased. The *duration* of the vasoconstriction usually increased slightly, rarely more than five seconds. The *frequency* of vasoconstriction, however, increased progressively so that the dilated phase intervened only by fractions of one second between the constricted phases. A virtually persistent ischemia of the arterial segment of the capillary loop was therefore produced, the duration of which depended upon the quantity of epinephrine or nor-epinephrine injected. The ischemia of the arterial segments of the capillary loops produced by the injection of epinephrine or nor-epinephrine could be distinguished from that occurring as the result of normal vasomotion by these characteristics: *1*) the ischemia began within the expected circulation time of arm to finger, 15 to 22 seconds; *2*) the ischemia occurred concomitantly in the adjacent capillary loops; *3*) the rate of blood flow through the capillary loops increased

[1] Hydrochloride salt (extract of adrenal medulla; supplied by Parke, Davis & Co.).

[2] Bitartrate salt (synthetic; supplied by Winthrop-Stearns, Inc.).

[3] Bitartrate salt (synthetic; supplied by Winthrop-Stearns, Inc.).

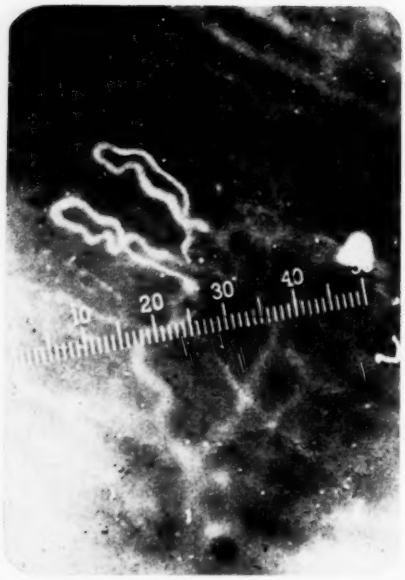

FIG. 1. CAPILLARY BED OF THE NAILFOLD OF A PATIENT WITH NORMAL BLOOD PRESSURE (80×)

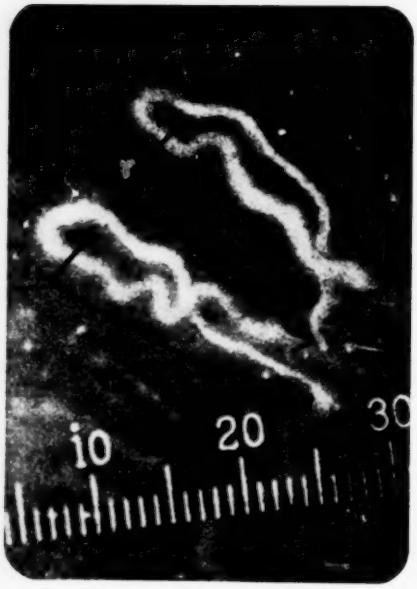

FIG. 2. TWO CAPILLARY LOOPS TAKEN FROM FIGURE 1
Arrows indicate the arterial segments (160×).

immediately prior to the development of the ischemia (this was not observed during normal vasomotion); *4*) the ischemia was interrupted by frequent momentary periods of blood flow; *5*) the shortest duration of ischemia selected for the endpoint of the titration, 15 seconds, was usually five to 20 times longer than that observed during the control period of observation; *6*) the ischemia was reproducible when the same quantity of epinephrine or nor-epinephrine was reinjected after a five minute interval.

FIG. 3. REACTION FOLLOWING 1.5 GAMMA OF EPINEPHRINE, I.V.
Arrows indicate junction of arterial segments with venous segments (160×).

It was usually impossible to visualize clearly the arterial system proximal to the terminal capillary loops. The possibility that the ischemia following the injection of epinephrine or nor-epinephrine was due to constriction of the parent metarteriole and not directly to constriction of the arterial segments of the capillary loops could not be excluded.

The capillary bed of the nailfold of the finger of a patient with normal blood pressure is reproduced in Figure 1, a microphotograph magnified

TABLE I

Threshold reactivity of the arterial segments of the capillary loops of the nailfold in patients with normal blood pressure

	Blood pressure	Pulse rate	Threshold level	Duration of effect	Drug*	Major diagnosis	Age
	mm. Hg	*per min.*	*gamma*	*seconds*			*years*
1	130/58	78	1.5	15	E	Pulmonary fibrosis and emphysema	59
2	120/68	72	2.25	25	E	Diabetes mellitus	46
			1.5	25	E1		46
3	112/70	80	2.0	25	E	Pernicious anemia	49
4	122/70	88	1.5	25	E	Post pneumonia	39
5	130/70	76	1.5	20	E	Pulmonary fibrosis and emphysema	75
6	140/70	78	1.75	20	E	Old cerebrovascular accident	75
7	148/70	78	1.5	20	E	Cerebral atherosclerosis	62
8	130/74	80	1.5	30	E	Diabetes mellitus	72
9	156/76	72	1.5	20	E	Cerebral atherosclerosis	73
10	130/80	106	1.0	20	E	Lymphosarcoma	47
11	150/67	92	1.25	30	E1	Pulmonary fibrosis and emphysema	59
12	136/68	80	1.5	30	N	Arteriosclerotic heart disease, III C	74
13	150/74	96	1.25	15	N	Old cerebrovascular accident	62
14	120/60	72	1.25	30	N	Cerebral atherosclerosis	64
15	110/76	80	1.5	35	N	Paget's disease of bone	57
16	160/86	88	1.25	30	N	Schizophrenia	55
17	120/76	80	1.75	15	N	Old cerebrovascular accident	76

Mean = 1.51 gamma.
Standard deviation = 0.30 gamma.
Standard error = 0.073 gamma.
*Epinephrine—E; Levo epinephrine—E1; Levo nor-epinephrine—N.

TABLE II

Threshold reactivity of the arterial segments of the capillary loops of the nailfold in patients with essential hypertension

	Blood pressure	Pulse rate	Threshold level	Duration of effect	Drug*	Other diagnosis	Age
	mm. Hg	*per min.*	*gamma*	*seconds*			*years*
1	150/100	80	0.5	30	E		70
2	150/100	88	0.75	35	E	Diabetes mellitus	40
3	210/100	84	0.75	30	E	Diabetes mellitus	50
4	150/110	76	0.75	60	E1	Recent cerebrovascular accident	65
5	170/90	64	0.75	45	E	Old cerebrovascular accident	75
	140/70†	72	1.75	20	E		
6	212/90	72	0.35	60	N	Cerebral atherosclerosis	80
7	190/90	100	0.25	25	N	Chronic bronchitis	59
8	150/92	72	0.25	25	N	Old myocardial infarction	66
			0.50	60	N		
9	130/92	80	0.35	60	N		52
10	160/94	88	0.25	30	N	Arteriosclerotic heart disease, III C	74
	136/68†	80	1.5	30	N		
11	190/110	80	0.35	20	N	Recent cerebrovascular accident	60
12	180/130	80	0.15	30	N		50
13	140/100	88	0.25	60	N		40
14	210/110	76	0.25	60	N	Old cerebrovascular accident	70
15	230/110	88	0.6	20	E1	Old cerebrovascular accident	74
			0.4	20	N		
16	190/88	72	0.75	15	E1	Cerebral atherosclerosis	68
			0.4	15	N		
17	200/100	88	0.5	30	E1		69
			0.5	20	N		
18	140/108	76	0.5	20	E1		48
			0.3	20	N		
			0.5	90	N		
19	190/90	86	0.5	20	N	Diabetes mellitus	68
			0.75	60	N		

Mean = 0.45 gamma.
Standard deviation = 0.17 gamma.
Standard error = 0.036 gamma.
* Epinephrine—E; Levo epinephrine—E1; Levo nor-epinephrine—N.
† Same case as the preceding one examined two and three weeks later, respectively.

TABLE III

Threshold reactivity of the arterial segments of the capillary loops of the nailfold in patients with inadequate capillary blood flow

Blood pressure	Pulse rate	Threshold level	Duration of effect	Drug*	Skin temp.	Skin color	Rate of flow	Circ. time	Sludge	Major diagnosis	Age
mm. Hg	*per min.*	*gamma*	*secs.*					*secs.*			*years*
118/60	120	10.0	10	E	Cold	Pale	Slow	23	3×	Pneumonia	39
122/70†	88	1.5	25	E	Warm	Pink	Rapid	15	1×	Convalescence	
96/76	86	5.0	20	E	Cool	Pink	Slow	28	3×	Cor pulmonale	60
100/78	76	4.0	25	E1	Cool	Cyanotic	Slow	26	3×	Cor pulmonale	62
		4.0	30	N				28			
110/80	100	7.5	20	N	Cool	Cyanotic	Slow	23	2×	Arteriosclerotic heart disease, IV E	68
126/80	100	5.0	15	N	Cool	Cyanotic	Slow	25	3×	Intestinal obstruction	80
		7.5	60	N							
80/60	120	5.0	40	N	Cool	Cyanotic	Slow	24	3×	Decompensated cirrhosis	65
160/80	76	20.0	20	N	Cold	Pale	Very slow	26	3×	Old cerebrovascular accident	55
96/60	80	4.0	30	E	Warm	Cyanotic	Slow	24	2×	Parkinsonism	65
110/60	74	2.5	30	E	Cool	Cyanotic	Slow	28	3×	Cerebral atherosclerosis	90
136/60	80	2.5	20	E	Cool	Cyanotic	Slow	28	3×	Cerebral atherosclerosis	86

* Epinephrine—E; Levo epinephrine—E1; Levo nor-epinephrine—N.
† Same case as the preceding one examined ten days later.

80 times. Figure 2 is a higher power photograph, magnified 160 times, of two of the capillary loops with arterial segments approximately 8 microns in diameter. The successive injection of 0.25, 0.50, and 0.75 gamma of epinephrine at five minute intervals produced no visible effect. The injection of 1.0 and 1.25 gamma of epinephrine each produced a transient increase in the vasomotion process but the capillary ischemia persisted no longer than eight and ten seconds respectively. Eighteen seconds following the intravenous administration of 1.5 gamma of epinephrine, constriction of the arterial segments of the loops occurred as seen in Figure 3, which persisted more than 15 seconds. This dose, 1.5 gamma, indicated the "threshold" quantity and was recorded as the endpoint.

The threshold capillary reactivities of patients with different blood pressures and adequate capillary blood flow are recorded in Tables I and II. The patients are divided into two major groups, those with normal blood pressure (Table I) and those with essential hypertension (Table II). For both epinephrine and nor-epinephrine, the threshold reactivity of the capillary bed in the nailfold of 17 individuals with normal blood pressure ranged from 1.0 to 2.25 gammas, with a mean of 1.51 gammas and a standard deviation of 0.30 gamma (Table I). The threshold reactivity of the capillary bed of 19 individuals with essential hypertension ranged from 0.5 to 0.75 gamma, with a mean of 0.65 gamma and a standard deviation of 0.12 gamma for epinephrine; and for nor-epinephrine from 0.15 to 0.50 gamma, with a mean of 0.34 gamma and a standard deviation of 0.09 gamma (Table II).

When capillary blood flow was inadequate, as indicated by coolness, pallor, or cyanosis of the skin, slowing of the capillary blood flow, clumping of the red blood cells, and prolongation of the circulation time, there was a wide range of "apparent" reactivity. Thus the threshold reactivity of the capillary bed in the nailfold of 10 patients with evidence of inadequate capillary flow ranged from 2.5 to 20.0 gammas for both epinephrine and nor-epinephrine (Table III).

DISCUSSION

The minute vascular bed of the nailfold is sensitive to circulating epinephrine and nor-epinephrine. The threshold reactivity of the arterial segments of these capillary loops can be visually "titrated" with epinephrine injected intravenously, and the results fall within a fairly narrow range, provided there is no interference with the blood flow to the capillary bed.

In the presence of an adequate capillary blood flow, the arterial segments of the terminal capillary loops of the nailfold of patients with essential hypertension exhibit a hyper-reactivity to circulating epinephrine and nor-epinephrine when compared with the capillary loops of subjects with normal

blood pressures ($t = 13$). The arterial segments of the capillary loops of the nailfold of patients with normal blood pressure appear to be equally reactive to epinephrine and nor-epinephrine ($t = 1.1$), whereas the capillary loops of patients with essential hypertension appear more reactive to nor-epinephrine than to epinephrine ($t = 6.4$).

The mechanism of the hyper-reactivity in patients with hypertensive vascular disease is conjectural. A small quantity of epinephrine is injected intravenously (0.1 to 0.8 cc.), acts rapidly upon the vascular bed, and is quickly inactivated. In disease states in which the effective concentration of the circulating epinephrine is reduced, the effect of the epinephrine is thereby altered. This will occur when the injected epinephrine is diluted to a greater volume (increased blood volume), or impeded from reaching the periphery (slow peripheral blood flow, low cardiac output). Changes in skin temperature would also alter capillary reactivity. Since the blood volume, the peripheral blood flow, the cardiac output, the circulation time, and the skin temperature are not significantly altered in hypertensive vascular disease (14–20), the difference in vascular reactivity to epinephrine probably is not attributable to these factors. The increased vascular reactivity in hypertensive vascular disease appears, justifiably, to be directly referable to the capillary bed. Epinephrine and nor-epinephrine appear to act upon the capillary bed by intensifying the normal vasomotion process. Vasomotion is usually increased in patients with essential hypertension. The hyper-reactivity of the arterial segments of the capillary bed to epinephrine and nor-epinephrine that occurs in essential hypertension may therefore be solely a reflection of this fundamental increase in vasoconstrictor activity.

It should again be stressed that the results presented apply only to the arterial segments of the terminal capillary loops of the nailfold of the finger.

SUMMARY AND CONCLUSIONS

1. The arterial segments of the capillary loops of the nailfold of the finger are sensitive to circulating epinephrine and nor-epinephrine. The threshold vasoconstrictor reactivity is fairly constant under the conditions outlined, and falls within a narrow range in persons with normal blood pressure and essential hypertension, when capillary blood flow is adequate.

2. The arterial segments of the terminal capillary loops in the nailfold of patients with essential hypertension are hyper-reactive to circulating epinephrine; this hyper-reactivity is even more marked to circulating nor-epinephrine.

3. These changes in epinephrine reactivity are referred directly to the capillary bed because the blood volume, the cardiac output, the peripheral blood flow, the circulation time, and the skin temperatures do not differ significantly from normal in individuals with essential hypertension.

4. Vasomotion of the capillary bed is intensified in patients with hypertensive vascular disease. The hyper-reactivity of the capillary bed to circulating epinephrine and nor-epinephrine in essential hypertension may be solely a reflection of this fundamental increase in vasoconstrictor activity.

5. The results and conclusions apply only to the capillary loops of the nailfold of the finger when the capillary blood flow is adequate.

REFERENCES

1. Gordon, W., and Levitt, G., Blood pressure changes in normals and in hypertensives after intravenous epinephrine and histamine. J. Clin. Invest., 1935, **14**, 367.
2. Pickering, G. W., and Kissin, M., The effects of adrenaline and of cold on the blood pressure in human hypertension. Clin. Sc., 1936, **2**, 201.
3. Fatherree, T. J., and Hines, E. A., Jr., The blood pressure response to epinephrine administered intravenously to subjects with normal blood pressure and to patients with essential hypertension. Am. Heart J., 1938, **16**, 66.
4. Judson, W. E., Culbertson, J. W., Tinsley, C. M., Litter, J., and Wilkins, R. W., The comparative effects of small intravenous doses of epinephrine upon arterial pressure and pulse rate in normotensive subjects and in hypertensive patients before and after thoracolumbar sympathectomy. J. Clin. Invest., 1950, **29**, 1405.
5. Judson, W. E., Epstein, F. H., and Wilkins, R. W., The comparative effects of small intravenous doses of *l*-nor-epinephrine upon arterial pressure and pulse rate in normotensive subjects and in hypertensive patients before and after thorocolumbar sympathectomy. J. Clin. Invest., 1950, **29**, 1414.
6. Goldenberg, M., Pines, K. L., Baldwin, E. de F., Greene, D. G., and Roh, C. E., The hemodynamic response of man to nor-epinephrine and epinephrine and its relation to the problem of hypertension. Am. J. Med., 1948, **5**, 792.

7. Clough, P. W., A study of the cardiovascular reaction to epinephrine. Epinephrine sensitiveness in patients with hypertension. Bull. Johns Hopkins Hosp., 1920, **31**, 266.
8. Lee, R. E., and Holze, E. A., The peripheral vascular system in the bulbar conjunctiva of young normotensive adults at rest. J. Clin. Invest., 1950, **29**, 146.
9. Lee, R. E., and Holze, E. A., Peripheral vascular hemodynamics in the bulbar conjunctiva of subjects with hypertensive vascular disease. J. Clin. Invest., 1951, **30**, 539.
10. Lewis, T., The blood vessels of the human skin and their responses. Shaw, London, 1927, 322 pp.
11. Zweifach, B. W., The character and distribution of the blood capillaries. Anat. Rec., 1939, **73**, 475.
12. Chambers, R., and Zweifach, B. W., Topography and function of the mesenteric capillary circulation. Am. J. Anat., 1944, **75**, 173.
13. Chambers, R., and Zweifach, B. W., Funtional activity of the blood capillary bed, with special reference to visceral tissue. Ann. New York Acad. Sci., 1946, **46**, 683.
14. Allen, E. V., Barker, N. W., Hines, E. A., Peripheral Vascular Diseases. W. B. Saunders Co., Philadelphia, 1946.
15. Prinzmetal, M., and Wilson, C., The nature of the peripheral resistance in arterial hypertension with special reference to the vasomotor system. J. Clin. Invest., 1936, **15**, 63.
16. Goldring, W., and Chasis, H., Hypertension and Hypertensive Diseases. The Commonwealth Fund, New York, 1944.
17. Pickering, G. W., The peripheral resistance in persistent arterial hypertension. Clin. Sc., 1936, **2**, 209.
18. Steele, J. M., and Kirk, E., The significance of the vessels of the skin in essential hypertension. J. Clin. Invest., 1934, **13**, 895.
19. Stewart, H. J., Evans, W. F., Haskell, H. S., and Brown, H., The peripheral blood flow and rectal and skin temperatures in hypertension. Am. Heart J., 1946, **31**, 617.
20. Blumgart, H. L., and Weiss, S., Studies on the velocity of blood flow. J. Clin. Invest., 1927, **4**, 173.

THYROID FUNCTION IN NEPHROSIS [1]

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(Submitted for publication March 9, 1952; accepted June 20, 1952)

Since hypometabolism and hypercholesterolemia are common to both hypothyroidism and nephrosis, it was postulated that thyroid activity was subnormal in nephrotic patients. However, Epstein found that remarkably large doses of thyroid hormone could be given to nephrotic patients without inducing hypermetabolism (1). Such tolerance to thyroid medication is unusual among patients with true hypothyroidism. It has therefore been suggested that the hypometabolism and increased serum cholesterol in nephrosis are due not to decreased thyroid function but to other causes, as yet unknown (2).

The recent demonstration that the concentration of protein-bound iodine in the serum is reduced in nephrosis (3) has once more raised the question of whether thyroid function is impaired in this syndrome. The present study is an attempt to answer this question.

MATERIALS AND METHODS

The subjects for this study were patients in the active phase of the nephrotic syndrome with hypoproteinemia, proteinuria, and edema. All were hospitalized, eight at the Peter Bent Brigham Hospital, eight at the Presbyterian Hospital, and three at the Massachusetts General Hospital. The sex, age, and type of nephrosis are listed in Table I.

The concentration of protein-bound iodine in the serum was determined by the method of Man, Smirnow, Gildea, and Peters (4). In certain patients the same method was used for the determination of protein-bound iodine in urine. The volume of urine used for analysis was so chosen as to contain approximately 0.4 gm. of protein. The urinary protein was precipitated with acid zinc sulfate and sodium hydroxide, using the same quantities of these reagents as for 6 ml. of serum. In order to obtain complete precipitation, the pH of the suspension of precipitate was carefully adjusted to between 7.0 and 7.5. When the concentration of protein was small and the aliquot of urine correspondingly large, as much as two or three times the quantities of precipitating reagents were required to avoid highly disperse precipitates. Before analysis, the precipitates were washed thrice in doubly distilled water and centrifuged.

In most patients the ability of the thyroid gland to accumulate iodine was tested by the oral administration of a tracer dose (100 microcuries or less) of radioactive iodine. The isotope with a half-life of 8.0 days, I^{131}, was obtained from the Clinton Laboratories, Oak Ridge, Tennessee. It was administered orally without added carrier. In some patients the per cent of the dose accumulated by the thyroid gland in 24 or 48 hours (uptake) was estimated by *in vivo* measurements of radioactivity in the neck. The details of the techniques used have been described (5, 6). In other patients the rate of collection (accumulation gradient) was measured by the method of Stanley and Astwood as described by Perlmutter and Riggs (7). The accumulation gradient is defined as the slope of the approximately straight line obtained when the radioactivity over the thyroid gland, expressed as counts per second, is plotted on the ordinate, against the square root of time in minutes on the abscissa.

In certain experiments thyrotropic hormone [3] was employed. Two preparations, one assaying 2.6 Evans units per mgm. and the other 4.5 Evans units per mgm., were used. The preparations were dissolved in physiological saline and injected intramuscularly in a dose of 15 mgm. every six hours for two days. The design of these experiments and of others in which d,l-thyroxine was injected intravenously will be described below under "Results."

RESULTS

The serum protein-bound iodine is subnormal in nephrosis. The concentration of serum protein-bound iodine in 16 patients with the nephrotic syndrome was distinctly subnormal: below 3.0 μg. % in 13, borderline (3.0 to 3.4 μg. %) in two, and normal (3.5 to 7.0 μg. %) in only one (Table I). The mean was 2.2 μg. %.

The ability of the thyroid gland to collect iodine

[1] This work was supported in part by funds received from the Eugene Higgins Trust, the Commonwealth Fund, and the National Research Council, Committee on Research on Endocrinology.

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[3] The thyrotropic hormone was kindly supplied to us by Armour and Co., Chicago, Ill.

TABLE I

Laboratory data in 19 nephrotic patients

Patient	Sex	Age	Diagnosis	Serum protein gm. %		Serum protein-bound iodine	Radioiodine		Basal metabolic rate	Serum cholesterol
				Total	Albumin		Uptake	Accumulation gradient*		
						%				mgm. %
T. E.	M	9	Nephrosis	—	1.5	1.3				400
T. K.	M	12	Nephrosis	—	3.0	3.1				425
C. L.	F	22	Nephrosis	3.7	2.2	1.8		18.0	−10%	335
J. P.	M	14	Nephrosis	3.8	1.5	2.2		9.4	−4%	474
D. H.	F	48	Nephrosis	2.5	1.0	1.7		12.2	−39%	340
S. H.	F	15	Nephrosis	3.0	1.7	2.0	32%†		−25%	750
A. M.	M	18	Nephrosis	3.2	1.5	1.8	40%†		−22%	600
F. C.	M	17	Nephrosis	3.5	2.2		55%†	19.0	−27%	316
J. C.	M	38	Nephrosis	3.4	2.2	1.8	26%†	8.0	−36%	492
G. D.	M	18	Nephrosis	3.2	0.9	0.9			−26%	755
P. O.	M	14	Chr. glom. nephrotic	5.7	2.3	3.4		5.0	−15%	176
H. D.	M	26	Chr. glom. nephrotic	3.8	2.0	1.5		0.7		595
J. H.	M	62	Chr. glom. nephrotic	3.6	2.5	2.0	75%‡			312
J. K.	M	34	Chr. glom. nephrotic	3.3	2.0	2.2	44%‡		−27%	510
M. R.	M	11	Chr. glom. nephrotic	3.7	1.6	2.1	25%†		−35%	716
F. R.	F	32	Lupus nephrosis	3.4	2.0	5.2	38%†	10.2	0%	329
V. F.	F	35	Lupus nephrosis	3.8	2.0		44%†	14.0	−10%	200
J. M.	F	20	Tridione nephrosis	3.0	1.9		38%†	10.0	−19%	562
F. A.	M	30	Amyloid nephrosis	4.3	2.0	1.9	71%‡		−26%	329
Mean		25		3.6	1.9	2.2		10.6	−21%	453

* Slope of line obtained when the counts per minute over the thyroid gland are plotted as the ordinate against the square root of the time in minutes as the abscissa. Normal range: approximately 0.4 to 10 (7).

† Per cent of a tracer dose of I^{131} in the thyroid as estimated from *in vivo* measurements 24 hours after administration. Normal range: 10% to 40% (5).

‡ Per cent of a tracer dose of I^{131} in the thyroid as estimated from *in vivo* measurements over the neck 48 hours after administration. Normal range: 17% to 58% (6). The urinary excretion of I^{131} was also measured in two patients: F.A., 15.2% in the first 24 hours, 1.7% in the second 24 hours; J.K., 35.0% in the first 24 hours, 11.4% in the second 24 hours.

is unimpaired in nephrotic patients. In 16 nephrotic patients the avidity of the thyroid gland for iodide was tested with tracer doses of I^{131} (Table I). In some patients the *rate* of collection (accumulation gradient) was measured, in others the percentage of the dose retained by the thyroid gland 24 or 48 hours after administration of radioactive iodine was determined. Both measurements were used in five patients. With but one exception (patient H. D.) the accumulation of iodide was rapid. In several of the patients the accumulation gradient was actually greater than in most normal subjects. Similarly the per cent of a tracer dose retained by the thyroid gland was either normal or, in patients J. H., F. A., R. C., V. F., greater than normal.

The basal metabolic rate tends to be low, and the serum cholesterol high in nephrosis. These well established facts are illustrated by the data in the last two columns of Table I. In 15 patients the mean BMR was − 21%. Ten of the 15 values were below − 15%. The mean serum cholesterol was 453 mgm. %; 17 of the 19 values were above 300 mgm. %.

Protein-bound iodine is lost in the urine of nephrotic patients. In four patients the urinary excretion of protein-bound iodine was measured during 24 hour periods (Table II). It varied considerably from patient to patient without any obvious relation to the degree of proteinuria. Thus patient D. H. excreted an average of 7.2 gm. of protein and 6.0 μg. of protein-bound iodine in 24 hours, while patient J. P. excreted 5.8 gm. of protein and 31.8 μg. of protein-bound iodine over the same period. In all four patients the amount of protein-bound iodine per gram of urinary protein was equal to or greater than the amount of protein-bound iodine per gram of serum protein. The ratio of these two is given in the last column of Table II under the heading "U"/"S."

Urinary protein-bound iodine increases when the serum protein-bound iodine increases. In two

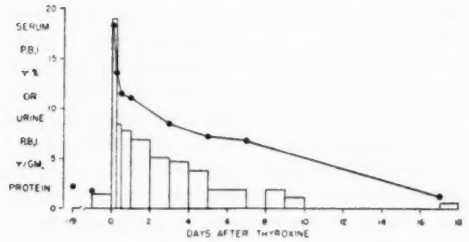

FIG. 1. NEPHROTIC PATIENT C. L., FEMALE. AGE, 22. BODY WEIGHT, 50.8 KGM.

At zero time, 7 mgm. of d,l-thyroxine were injected intravenously. The serum protein-bound iodine is represented by the solid circles. The concentration of protein-bound iodine in the urine per gram of urinary protein is indicated by the open bars.

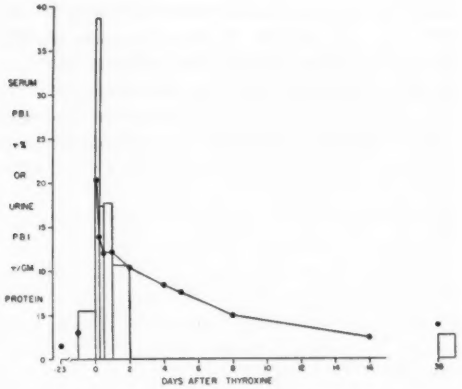

FIG. 2. NEPHROTIC PATIENT J. P., FEMALE. AGE, 13. BODY WEIGHT, 36.4 KGM.

At zero time, 5 mgm. of d,l-thyroxine were injected intravenously. The serum protein-bound iodine is represented by the solid circles. The concentration of protein-bound iodine in the urine per gram of urinary protein is indicated by the open bars.

nephrotic patients the serum protein-bound iodine was deliberately increased by the intravenous administration of d,l-thyroxine in a dose of 140 μg. per Kgm. of body weight (Figures 1 and 2). The excretion of protein-bound iodine in the urine rose immediately and then decreased as the serum protein-bound iodine fell towards the initial level. There was a better correlation between the *iodine per gram of urine protein* and the concentration of protein-bound iodine in the serum than between the *total quantity* of protein-bound iodine excreted and the serum protein-bound iodine. This suggests that the appearance of protein-bound iodine in the urine was due at least in part to the excretion of protein to which iodine was already bound, and not simply to an excretion of free thyroxin which subsequently became bound to the protein in the urine.

The rate of decrease of protein-bound iodine in the serum is not abnormally rapid after the administration of thyroxine to nephrotic patients. The time required for the serum protein-bound

TABLE II

Loss of protein-bound iodine in the urine in untreated patients with nephrosis

Patient	Serum total protein	Serum protein-bound iodine	Serum protein-bound iodine	Urine total protein per 24 hrs.	Urine protein-bound iodine	Urine protein-bound iodine	"U"/"S"
	gm. %	μg. %	μg. per gm. protein (S)	gm.	μg. per 24 hrs.	μg. per gm. protein (U)	
M. R.	3.7	1.8	0.49	12.9	16.2	1.25	
		2.3	0.62	22.2	22.7	1.02	
Mean	3.7	2.0	0.56	17.5	19.4	1.14	2.04
D. H.	2.5	1.7	0.68	7.3	3.6	0.50	
		2.5	1.00	8.3	6.6	0.79	
				6.0	7.9	1.31	
Mean	2.5	2.1	0.84	7.2	6.0	0.87	1.04
J. P.	3.8	3.0	0.79	5.8	31.8	5.48	6.94
C. L.	3.7	1.8	0.49	24.2	36.1	1.49	3.04
Mean of four pts.	3.4	2.2	0.67	13.7	23.3	2.24	3.26

iodine to return to initial levels following the intravenous injection of d,l-thyroxine was studied in two nephrotic patients, two patients with untreated myxedema, and one normal subject. In Figure 3, the increase of the serum protein-bound iodine above the initial control concentration is plotted on a logarithmic scale against time on an arithmetic scale. The curves are quite similar in shape to the curve for radioactive d,l-thyroxine in the serum of a cretin studied by Albert and Keating (8). The relatively rapid initial decrease of the concentration during the first 12 hours was probably due to the distribution of thyroxine from the blood stream to the tissues. The subsequent slow decline of concentration was presumably dependent upon the rate of utilization and excretion of thyroxine after distribution equilibrium had been attained. This second rate of decline was no more rapid in the nephrotic patients than in the normal subject. The time needed for the concentration of protein-bound iodine to decrease 50% was approximately 4.3 days both for the normal subject and for the nephrotic patients. This agrees well with the biological half-life of radioactive thyroxine, 3.8 days, in Albert's and Keating's patient with *treated* hypothyroidism. In contrast, the time required for a 50% decrease in concentration in our patients with *untreated* hypothyroidism was 8.3 days.

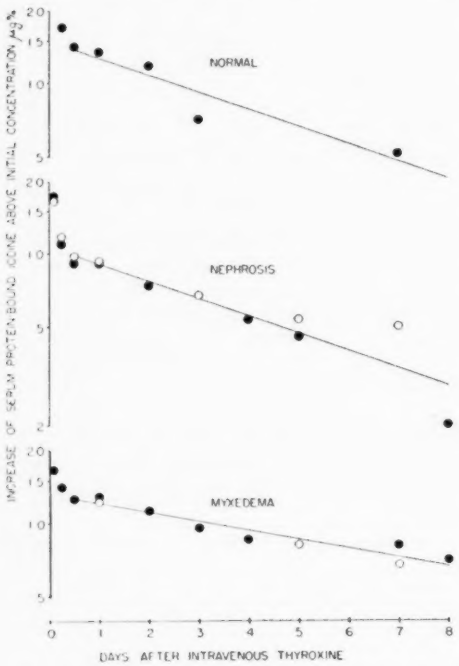

FIG. 3. THE RATE OF DECREASE OF SERUM PROTEIN-BOUND IODINE AFTER THE INTRAVENOUS ADMINISTRATION OF D,L-THYROXINE

The increase in serum protein-bound iodine above the initial control concentration is plotted on a logarithmic scale against time in days after the injection of thyroxine. In determining the straight lines by the method of least squares, the points at times less than 12 hours have been disregarded, and the data from patients with the same diagnosis have been pooled.

Top curve: Normal male, age 20, dose of thyroxine 10 mgm. *Middle curve:* Open circles = nephrotic patient C. L., female, age 22, dose of thyroxine 7 mgm.; solid circles = nephrotic patient J. P., female, age 13, dose of thyroxine 5 mgm. *Bottom curve:* Open circles = patient with pituitary myxedema, age 48, dose of thyroxine 5 mgm.; solid circles = patient with spontaneous myxedema, female, age 71, dose of thyroxine 5 mgm. The slopes of the curves indicate the following times for the concentration to decrease by 50%: Normal, 4.3 days; Nephrosis, 4.3 days; Myxedema, 8.3 days.

Thyroid activity in nephrotic patients decreases in response to an excess of thyroid hormone. In the two nephrotic patients and the one normal subject given thyroxine, the avidity of the thyroid gland for iodine as measured by the accumulation gradient decreased markedly after the administration of thyroxine (Table III). In patient J. P. the gradient decreased from 9.4 before thyroxine to 0.7, seven days after thyroxine, and was still only 2.5 one month after thyroxine. Presumably this decrease was due to inhibition of the output of thyrotropic hormone by the anterior pituitary gland in response to an excessive supply of thyroid hormone (9).

In nephrosis the thyroid gland is able to respond to thyrotropic hormone. Two patients with ne-

TABLE III

Effect of I.V. thyroxine upon I^{131} accumulation gradient

Subject	Gradient before treatment	Gradient 7 days after treatment
Nephrotic J. P.	9.4	0.7
Nephrotic C. L.	18.0	3.0
Normal R. W.	11.0	3.0

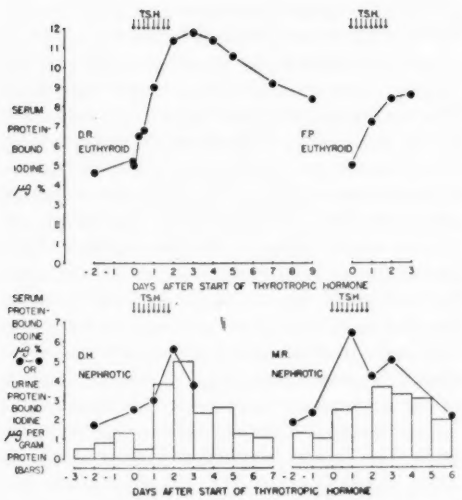
TSH
TSH
SERUM PROTEIN-BOUND IODINE μg %
D.R. EUTHYROID
F.P. EUTHYROID
DAYS AFTER START OF THYROTROPIC HORMONE
SERUM PROTEIN-BOUND IODINE μg % OR URINE PROTEIN-BOUND IODINE μg PER GRAM PROTEIN (BARS)
D.H. NEPHROTIC
M.R. NEPHROTIC
DAYS AFTER START OF THYROTROPIC HORMONE

FIG. 4. THE RESPONSE OF NEPHROTIC PATIENTS AND OF NORMAL SUBJECTS TO THYROTROPIC HORMONE

Each of the arrows marked "TSH" indicates the intramuscular injection of 15 mgm. of Armour's thyrotropic hormone. The preparation administered to D. R. contained 4.5 Evans units per mgm. The preparation used in the other three subjects contained 2.6 Evans units per mgm.

D. R., normal male, age 37. F. P., euthyroid male, age 66, recovering from an abdominal operation. D. H., nephrotic female, age 48. M. R., nephrotic male, age 11.

phrosis were given 15 mgm. of Armour's thyrotropic hormone intramuscularly every six hours for two days (Figure 4). In both patients the serum protein-bound iodine and the urinary protein-bound iodine rose significantly during the administration of thyrotropic hormone. The response of the serum protein-bound iodine in two euthyroid male subjects given thyrotropic hormone is also depicted in Figure 4. The thyrotropic hormone given to one of the normal subjects, D. R., was a different preparation from that used in the nephrotic patients, although in terms of units the dose (approximately 4.5 Evans units per Kgm. of edema-free body weight per day) was about the same as in patient M. R. In the normal subjects the elevation in protein-bound iodine appeared to be sustained longer than in the nephrotic patients. However, since little is known about normal variations in the response of different subjects to thyrotropic hormone, the significance of this difference between normal and nephrotic cannot be interpreted.

A comparatively brief period of treatment with 1-methyl-2-mercaptoimidazole may inhibit the response of the nephrotic patient to thyrotropic hormone. The ability of thyrotropic hormone to elicit a rise of serum protein-bound iodine during a brief period of treatment with 1-methyl-2-mercaptoimidazole was tested in one patient with nephrosis and in one euthyroid patient (Figure 5). In both patients, 10 mgm. of 1-methyl-2-mercaptoimidazole, three times a day, produced an almost complete block of thyroid hormone synthesis as indicated by a greatly diminished uptake of radioactive iodine. During the first eight or nine days of treatment, the serum protein-bound iodine did not change significantly in the patient with nephrosis, and fell only slightly in the euthyroid patient. While 1-methyl-2-mercaptoimidazole was continued, each subject was given 15 mgm. of the same preparation of Armour's thyrotropic hormone intramuscularly every six hours for two days. The serum protein-bound iodine in the euthyroid patient promptly rose from 3.4 to 7.9 μg. %. However, in the nephrotic patient, it not only failed to rise appreciably, but actually fell somewhat the day after thyrotropic hormone was discontinued. These observations suggest that when the synthesis of new hormone was blocked for a few days,

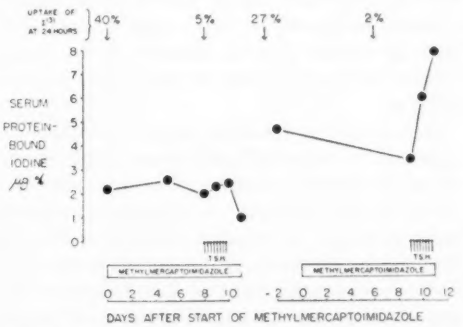

FIG. 5. THE RESPONSE OF A NEPHROTIC SUBJECT (LEFT) AND A EUTHYROID SUBJECT (RIGHT) TO THE ADMINISTRATION OF THYROTROPIC HORMONE DURING A BRIEF PERIOD OF TREATMENT WITH 1-METHYL-2-MERCAPTOIMIDAZOLE

The dose of 1-methyl-2-mercaptoimidazole was 10 mgm. three times a day. Each arrow marked "TSH" indicates the intramuscular injection of 15 mgm. of Armour's thyrotropic hormone containing 2.6 Evans units per mgm.

too little hormone remained in the thyroid gland of the nephrotic patient to permit a rise of the serum protein-bound iodine in response to thyrotropic hormone.

The thyroid gland is not usually enlarged in nephrosis. Thyroid enlargement was not noted in any of the patients in the present series.

DISCUSSION

The results of the present study suggest several hypotheses concerning thyroid function in nephrosis.

Although abnormal loss of thyroid hormone in the urine occurs, this finding does not of itself adequately explain all the observed facts. In four nephrotic patients the greatest loss of protein-bound iodine was 36 μg. in 24 hours. In two cases, Peters and Man observed a loss of 16 to 48 μg. (3). Since patients with myxedema require for replacement therapy about 100 μg. of iodine as l-thyroxine (10), and calculations in the euthyroid individual based upon radioactive iodine uptake and iodide excretion studies indicate a normal daily secretion of 50 μg. of hormonal iodine (11), it is apparent that significant losses of hormonal iodine may, but do not always, occur in the nephrotic. Further, in two patients following intravenously administered thyroxine, urinary loss in 48 hours was only 6.7 and 6.3% of the dose. These observations coupled with an apparently normal rate of disappearance of serum protein-bound iodine make it improbable that the urinary loss of hormone is the major problem with regard to the thyroid status in these patients.

If the loss of hormone should quantitatively represent an important mechanism depriving the tissues of thyroid substance, a compensatory increase in the secretion of pituitary thyrotropic hormone would be expected. Providing that the resulting hyperactivity of the thyroid gland could keep pace with the hormone loss, the concentration of serum protein-bound iodine would be restored to normal and, depending upon the degree of hyperactivity necessary for restoring this balance, thyroid enlargement might become evident. In fact, however, the concentration of protein-bound iodine in the serum remains low, and goiter has not been noted in patients with nephrosis. Thus, if this urinary loss were quantitatively significant one would have to postulate an additional pituitary or thyroid deficiency to explain the consistently low serum levels of hormone.

The possibility of inadequate secretion of thyrotropic hormone in nephrosis was considered. The avidity of the thyroid gland for radioactive iodine was normal or increased in all cases studied. Since there is good evidence that the capacity to accumulate iodide is dependent upon a normal supply of thyrotropic hormone, a pituitary deficiency seems unlikely. Further evidence that a normal thyrotropic hormone mechanism exists may be found in the response of the nephrotic to exogenously administered thyroxine, both in terms of a depression in radioactive iodine uptake and in the clinical tolerance to the drug. Finally, the behavior of the nephrotic subject to combined mercaptoimidazole and thyrotropic hormone therapy suggests that thyroid hormone stores are small and being turned over rapidly. A similar response has been reported in thyrotoxic patients (12). Since the ability of the thyroid gland to release thyroid hormone is also considered to be dependent upon the thyrotropic hormone, *a deficiency of thyrotropic activity in the nephrotic appears improbable.*

No indications of a specific thyroid gland abnormality or enlargement have been observed in the patients studied. The thyroid gland apparently can respond to exogenous thyrotropic hormone stimulation, though the quantitative aspects of the response as compared with the normal are difficult to evaluate. In addition, the thyroid gland can take up and accumulate radioactive iodine as well as or better than normal. The possibility that this latter avidity is associated with an iodine deficiency seems unreasonable in view of the abundant iodine supply in food and water in this region and the absence of an increased iodide clearance by the kidney. Indeed in J. K., in whom the urinary excretion of radioactive iodine was measured, it was slower than normal with a disproportionately large excretion in the second 24 hours (footnote, Table I). Further, iodide deficiency without some degree of thyroid enlargement would be extremely unusual.

Wolbach and Blackfan described extraordinary pathological changes in the thyroid glands of two children with nephrosis who came to autopsy (13). There was complete loss of colloid, desquamation and necrosis of the acinar epithelium, and increased

vascularity. Such extensive changes could hardly be compatible with normal thyroid function. Yet it seems likely that the histological picture described by Wolbach and Blackfan is exceptional. We are not aware of any subsequent report of similar pathological changes. Furthermore, no abnormalities of the thyroid gland were found among 10 patients with nephrosis who came to autopsy at the Babies Hospital in New York between 1935 and 1950.

It is clear, then, that a low serum protein-bound iodine must be explained despite the absence of obvious hypofunction of the thyroid or pituitary in nephrosis. Throughout the preceding discussion the tacit assumption has been made that the triad of low serum protein-bound iodine, low basal metabolic rate, and high serum cholesterol in nephrosis indicate a decreased supply of thyroid hormone to the tissues. This assumption may well be erroneous. Attention must be directed to the paucity of clinical evidence for hypothyroidism in nephrosis, to the differences between the lipemia of nephrosis and of myxedema, and to the tolerance of nephrotic patients for exogenous thyroid hormone which is certainly not characteristic of true hypothyroidism (3).

Careful consideration must therefore be given to the hypothesis that *thyroid function and the supply of hormone to the tissues in nephrosis may be normal, and that the low concentration of protein-bound iodine in the plasma is due to the change in concentration or binding capacity of the plasma proteins in nephrosis.* This hypothesis implies that when plasma protein is low, or is altered so that it is no longer able to bind thyroid hormone normally, the ability of the peripheral tissues to clear hormone from the blood stream increases. Although the circulating thyroid hormone, presumably thyroxine (14), is rather firmly bound to the proteins of plasma, it is quite possible that a fraction, undetectable by present analytical methods, is unbound. If there exists an equilibrium between protein, free thyroxine, and protein-bound thyroxine which follows the law of mass action, then a reduction in the concentration of plasma protein to which thyroxine is bound would necessitate an increase in the proportion of free thyroxine. If it be further assumed that thyroxine can traverse the cell wall and reach its site of action only in the unbound state, then an increase in the concentration of free thyroxine might favor a greater than normal rate of entry of hormone into the cells. *Hypoproteinemia would thus permit a normal supply of thyroid hormone to be carried to the tissues despite a decreased concentration of protein-bound hormone in the blood stream.*

Goldstein (15) has pointed out that the assumptions involved in this argument are not necessarily invalidated by failure to demonstrate dissociation of the thyroxine protein complex during prolonged dialysis *in vitro* (16). The occurrence of small quantities of free thyroxine in normal urine (17) suggests that traces of unbound thyroxine may indeed be present in plasma.

The hypothesis just advanced is analogous to the explanation offered by McLean and Hastings for the dependence of serum calcium on serum protein concentration (18). The parathyroid glands normally maintain a reasonably constant concentration of *ionized* calcium in the serum. When the concentration of ionized calcium is held constant, the concentration of *protein-bound* calcium varies directly with the protein concentration.

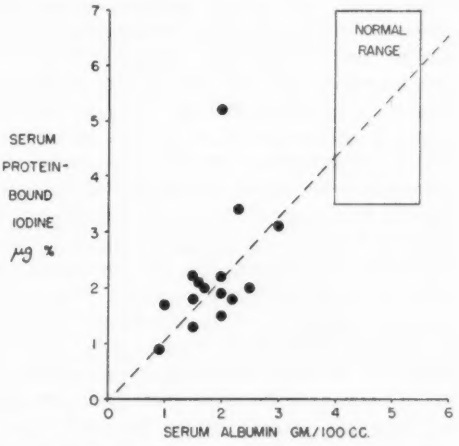

FIG. 6. THE RELATIONSHIP BETWEEN THE CONCENTRATIONS OF PROTEIN-BOUND IODINE AND OF CRUDE SERUM ALBUMIN IN THE SERUM OF PATIENTS WITH NEPHROSIS

Each circle represents a single patient. The broken line is intermediate between the lines of regression of albumin on iodine and of iodine on albumin. In calculating these regression lines by the method of least squares, the one circle within the normal range of protein-bound iodine was neglected. For comparison the approximate normal range is depicted as a rectangle.

Similarly, if the concentration of *free* thyroxine is normally kept within narrow limits by the homeostatic balance between thyroid and anterior pituitary, the concentration of *protein-bound* thyroxine should vary directly with the concentration of the thyroxine-binding groups of plasma proteins. There is evidence that most of these groups are in the albumin fraction when the usual clinical methods for the separation of serum protein into albumin and globulin are employed (16). In Figure 6, the concentration of protein-bound iodine in our patients with nephrosis has been plotted against the concentration of serum albumin. If the single normal value for protein-bound iodine (patient F. R.) is excluded, there is a highly significant correlation between these two variables. In the nephrotic patients, the ratio of mean serum albumin (in gm. %) to mean serum protein-bound iodine (in μg. %) was $1.7/1.9 = .89$. The ratio of the corresponding mean values for normal subjects is approximately $4.8/5.1 = .94$. The similarity of these ratios is consistent with the hypothesis under discussion.

The observed facts—the low serum protein-bound iodine, the normal uptake of radioactive iodine, the absence of goiter, the response to thyrotropic hormone, the lack of clinical evidence for hypothyroidism, and the tolerance of nephrotic patients to exogenous thyroid hormone—are all consistent with a euthyroid state in individuals with hypoproteinemia. However, the hypothesis by itself does not explain the failure of thyrotropic hormone to produce a rise in serum protein-bound iodine in the nephrotic patient treated with mercaptoimidazole (Figure 5), nor the increased avidity of the thyroid gland for iodine. These observations suggest that in nephrosis the quantity of hormone stored in the thyroid gland may be decreased. It seems probable that the continuous loss of hormone in the urine (both protein-bound and free) may gradually deplete the gland of preformed hormone. Such depletion must ultimately reduce the effective level of hormone in the serum so that a *slight compensatory increase in thyroid activity* would be required to restore the normal supply of hormone to the tissues. This state can be achieved without sufficient growth of the gland to produce a palpable goiter, but with an abnormally rapid and extensive uptake of radioactive iodine.

If, in nephrosis, the tissues actually receive an adequate supply of thyroid hormone despite a decreased concentration in the blood stream, deficient thyroid function cannot be held responsible for the subnormal metabolic rate. The frequent association of presumably normal thyroid activity and low basal metabolism in anorexia nervosa (3) has been noted. Similar discrepancies are often encountered among patients with partial hypopituitarism, hypogonadism (19) and among patients maintained on the Kempner rice diet (20). Adequate thyroid function seems to be necessary, but not sufficient, for the maintenance of a normal basal metabolic rate. It may be that in nephrosis the severe protein deficiency itself limits the basal rate of metabolism.

SUMMARY AND CONCLUSIONS

In 16 patients with active nephrosis the serum protein-bound iodine was low, and the serum cholesterol high. The basal metabolic rate was often subnormal even when calculated on the basis of edema-free weight. However, the uptake of radioactive iodine by the thyroid gland was normal or greater than normal, and there was a definite rise in the serum protein-bound iodine in response to thyrotropic hormone. In one patient this response was eliminated by treatment with mercaptoimidazole before and during the injection of thyrotropic hormone.

The results of this study support the supposition that thyroid function in nephrosis is essentially normal and that neither anterior pituitary failure nor inability of the thyroid gland to manufacture thyroid hormone in adequate amounts accounts for the low serum protein-bound iodine. Although protein-bound iodine was lost in the urine, it usually represented but a small portion of the estimated normal daily secretion of thyroid hormone. Furthermore, although the protein-bound iodine in the urine increased when the serum protein-bound iodine was raised by the intravenous administration of thyroxine, the urinary loss during the first two days amounted to only about 6% of the administered dose, and the rate of decrease of the serum protein-bound iodine after thyroxine was no greater than in a normal subject. It seems improbable that the urinary loss of hormone can by itself account for the subnormal concentration of protein-bound iodine in the serum.

Arguments are advanced in favor of the hypothesis that in nephrosis the decreased concentration of protein in the plasma accounts for the low serum protein-bound iodine and permits the transport and delivery of a normal supply of thyroid hormone to the tissues with a decreased concentration of hormone in the blood stream. In certain patients with nephrosis the thyroid gland may actually become somewhat hyperactive in order to compensate for the continuous loss of hormone in the urine.

The subnormal basal metabolic rate cannot be ascribed to hypothyroidism, but must be due to some other factor, perhaps the marked protein deficiency which occurs in nephrosis.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Dr. G. W. Thorn for his advice and suggestions.

REFERENCES

1. Epstein, A. A., Thyroid therapy and thyroid tolerance in chronic nephrosis. J.A.M.A., 1926, **87**, 913.
2. Leiter, L., Nephrosis. Medicine, 1931, **10**, 135.
3. Peters, J. P., and Man, E. B., The relation of albumin to precipitable iodine of serum. J. Clin. Invest., 1948, **27**, 397.
4. Man, E. B., Smirnow, A. E., Gildea, E. F., and Peters, J. P., Serum iodine fractions in hyperthyroidism. J. Clin. Invest., 1942, **21**, 773.
5. Werner, S. C., Quimby, E. H., and Schmidt, C., The use of tracer doses of radioactive iodine, I^{131}, in the study of normal and disordered thyroid function in man. J. Clin. Endocrinol., 1949, **9**, 342.
6. Skanse, B., Radioactive iodine in the diagnosis of thyroid disease. Acta med. Scandinav., Supp. 235, 1949, **136**.
7. Perlmutter, M., and Riggs, D. S., Thyroid collection of radioactive iodide and serum protein-bound iodine concentration in senescence, in hypothyroidism and in hypopituitarism. J. Clin. Endocrinol., 1949, **9**, 430.
8. Albert, A., and Keating, F. R., Jr., Metabolic studies with I^{131} labeled thyroid compounds. Comparison of the distribution and fate of radioactive d-l-thyroxine after oral and intravenous administration in the human. J. Clin. Endocrinol., 1949, **9**, 1406.
9. Greer, M. A., The effect on endogenous thyroid activity of feeding desiccated thyroid to normal human subjects. New England J. Med., 1951, **244**, 385.
10. Winkler, A. W., Riggs, D. S., and Man, E. B., Serum iodine in hypothyroidism before and during thyroid therapy. J. Clin. Invest., 1945, **24**, 732.
11. Stanbury, J. B., Brownell, G. L., Riggs, D. S., Perinetti, H., del Castillo, E., and Itoiz, J., The iodine-deficient human thyroid gland. A preliminary report. J. Clin. Endocrinol., 1952, **12**, 191.
12. Goldsmith, R. E., Stanbury, J. B., and Brownell, G. L., Effect of thyrotropin on the release of hormone from the human thyroid. J. Clin. Endocrinol., 1951, **11**, 1079.
13. Wolbach, S. B., and Blackfan, K. D., Clinical and pathological studies on tubular nephritis (nephrosis). Tr. A. Am. Physicians, 1929, **44**, 77.
14. Taurog, A., Chaikoff, I. L., and Tong, W., The nature of plasma iodine as revealed by filter paper partition chromatography. J. Biol. Chem., 1950, **184**, 99.
15. Goldstein, A., The interactions of drugs and plasma proteins. J. Pharmacol. & Exper. Therap., Part II, 1949, **95**, 102.
16. Riggs, D. S., Lavietes, P. H., and Man, E. B., Investigations on the nature of blood iodine. J. Biol. Chem., 1942, **143**, 363.
17. Rall, J. E., Iodine compounds in the blood and urine of man. J. Clin. Endocrinol., 1950, **10**, 996.
18. McLean, F. C., and Hastings, A. B., The state of calcium in the fluids of the body. I. The conditions affecting the ionization of calcium. J. Biol. Chem., 1935, **108**, 285.
19. Peters, J. P., German, W. J., and Man, E. B., Serum precipitable iodine in patients with tumors of or near the pituitary. J. Clin. Endocrinol., 1949, **9**, 1292.
20. Currens, J. H., Reid, E. A. S., MacLachlan, E. A., and Simeone, F. A., Metabolic effects of the rice diet in the treatment of hypertension. New England J. Med., 1951, **245**, 354.

THE EXTRARENAL CORRECTION OF ALKALOSIS ASSOCIATED WITH POTASSIUM DEFICIENCY [1]

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(Submitted for publication April 21, 1952; accepted June 20, 1952)

The role of the kidney in the regulation of the pH of extracellular fluids has been adequately described (1, 2). However, although the alterations in muscle composition accompanying changes in acid-base balance have been pointed out (3), quantitative evaluation of the effects of these changes in muscle composition on the acid-base equilibrium of extracellular fluids has received little attention. The determination of the precise role of kidney and of muscle cell in modifying extracellular composition has been handicapped by the difficulty in designing experiments which separate the influence of different organs and systems. The need for a "body-less kidney" or a "kidney-less body" in the study of this problem has been emphasized by Darrow (4).

The physiological basis for metabolic alkalosis associated with potassium deficiency has not been satisfactorily determined. On the basis of changes in renal potassium excretion during administration of the carbonic anhydrase inhibitor, 6063, Berliner, Kennedy, and Orloff (5) have postulated that alkalosis in potassium deficiency results from the excessive substitution of hydrogen and ammonium ions for sodium in the distal tubule of the kidney. In this way chloride is excreted without sodium and hypochloremic alkalosis of the extracellular fluid results.

This paper presents the findings in experiments designed to test this hypothesis by simultaneous measurement of renal excretion and analysis of serum and muscle of rats during recovery from potassium deficiency alkalosis.

PLAN OF EXPERIMENTS AND METHODS

Male albino rats, weighing 250–300 grams, were made alkalotic and potassium deficient in the course of 3½ weeks by the feeding of a potassium-deficient diet [4] and the intraperitoneal injection of 2 mg. of desoxycorticosterone acetate (DCA) [5] daily. An isotonic solution of sodium chloride and sodium acetate, with a molecular sodium to chloride ratio of 1.5/1.0, was substituted for drinking water and was the sole source of sodium and chloride.

Animals were then divided into two groups of nine animals each and transferred to individual metabolism cages. Distilled water was substituted for the salt solution, DCA injections were discontinued but the diet was not modified. The intake of food by each group was grossly the same although paired feeding was not carried out. Even large differences in food intake could not significantly affect the urinary excretion since the diet was devoid of sodium, potassium, and chloride and contained only small quantities of phosphorus, calcium, magnesium, and trace elements. After three days a control urine collection for 24 hours was obtained.

Group K then received 3 mM of KCl per kilogram of body weight twice daily, administered intraperitoneally as isotonic potassium chloride. Complete urine collections were obtained and analyzed each day. This dosage of potassium chloride approximates the maximum used in the repair of potassium deficiency in humans. Larger doses are poorly tolerated by the animals. Group Na received twice daily intraperitoneal injections of the equivalent amount of chloride as Group K in the form of isotonic sodium chloride and all urine was likewise collected and analyzed.

All cages, screens and funnels were treated with a silicone to facilitate rapid and complete drainage. All urine was collected under toluene and mineral oil. Feces were excluded from urine but were not analyzed. Other animals were treated similarly except that urine was not collected. These animals were sacrificed at the beginning of and at appropriate intervals throughout the

[1] Aided by research grants from the United States Public Health Service and the James Hudson Brown Memorial Fund of the Yale University School of Medicine.

[2] John and Mary R. Markle Scholar in Medical Science.

[3] Medical Research Fellow, Rotary International Foundation.

[4] Constituents per Kg. of Diet—dextrin 550 g., hydrogenated vegetable oil (Swiftning) 150 g., raw casein 100 g., vitaminized casein 100 g., corn oil 100 g., vitaminized corn oil 100 g., basic salts 5 g. (including calcium, phosphorus, iron, zinc, copper, manganese, magnesium).

[5] Percorten, supplied by Ciba, Inc., Summit, N. J., whose generosity made possible these studies.

experiment. Analyses of blood drawn from the abdominal aorta during ether anesthesia and of muscle after exsanguination were obtained on these animals as well as those studied in metabolism cages.

The methods of analysis are the same as were used earlier in this laboratory with the exception that sodium and potassium were determined by a Barclay internal standard flame photometer. The microdiffusion method of Conway (6) was used for analysis of ammonia in urine. The Beckman pH meter was used for the determination of pH and titratable acidity by titration with N/100 NaOH to pH 7.40. It should be pointed out that serum pH is subject to the largest temporary variations of the quantities measured because of alterations in respiratory activity associated with anesthesia and exsanguination. The carbon dioxide content and chloride concentrations are therefore more significant indicators in rats of disturbance of acid-base balance.

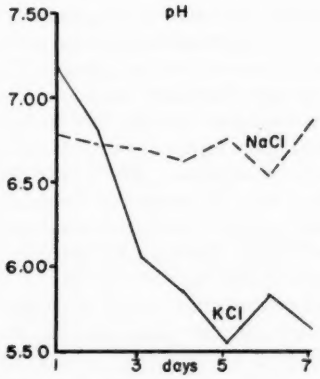

FIG. 1. AVERAGE URINE pH IN GROUPS K AND Na

RESULTS

Results are presented as the mean of each group since the variations among animals of the same group were small. The significant trends in urinary excretion which occurred during the course of the experiments were observed in every animal studied.

There was essentially no difference in mean renal excretion between Groups K and Na during the control period (day 1 in Figures 1–8). There was a marked difference in composition of urine between Groups K and Na after injections of potassium and sodium chloride were begun.

Group K excreted urine of lower pH and bicarbonate content and higher titratable acidity than Group Na. These findings became more marked as the experiment progressed (Figures 1, 2, 3).

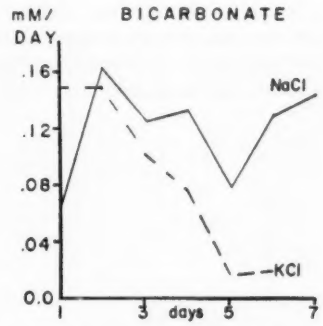

FIG. 2. AVERAGE BICARBONATE EXCRETION IN GROUPS K AND Na

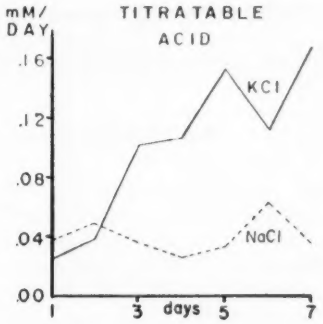

FIG. 3. AVERAGE TITRATABLE ACID EXCRETION IN GROUPS K AND Na

The ammonia excretion was somewhat greater in Group K than in Group Na (Figure 4). In Group K there was marked increase in the excre-

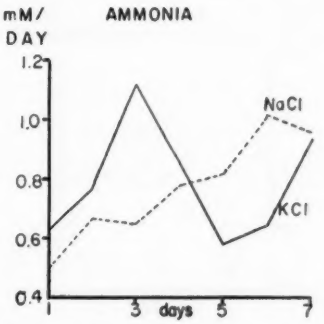

FIG. 4. AVERAGE AMMONIA EXCRETION IN GROUPS K AND Na

tion of sodium after the injection of 6 mM of potassium per kilogram of body weight, although no sodium had been taken for five days (Figure 5). This loss of sodium diminished after 18–24 mM/Kg. of potassium had been administered. In those animals receiving sodium chloride the excretion of sodium was almost equivalent to the administered sodium from the first day of the experiment (Figure 5).

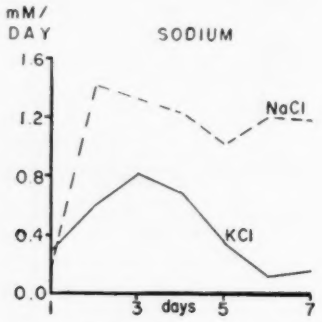

FIG. 5. AVERAGE SODIUM EXCRETION IN GROUPS K AND NA

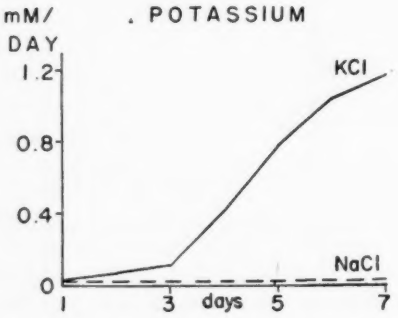

FIG. 6. AVERAGE POTASSIUM EXCRETION IN GROUPS K AND NA

The excretion of potassium by Group K did not increase significantly until 12 mM/Kg. of potassium had been injected (Figure 6) and it was not until 24 mM/Kg. had been administered that excretion of potassium approximated the intake. There was essentially no increase in potassium excretion in those animals given sodium chloride (Figure 6).

There was a fall in phosphorous excretion after the administration of 6 mM/Kg. of potassium but there was no consistent difference in the excretion of phosphorus between the two groups of animals (Figure 7). There was no uniform difference in Cl excretion between the two groups (Figure 8).

The average cumulative balance of potassium, sodium and chloride was calculated for each group of animals. The intake of each ion was that administered by injection. The loss of these ions in the feces was not included in the calculation of outgo and probably produces a small but significant error in the potassium balance only. In the potassium chloride treated animals the retention of potassium amounted to 20.2 mM/Kg. of body weight over six days and the loss of sodium amounted to 10.8 mM/Kg. of body weight. The retention of chloride was less than the sum of the retentions of sodium and potassium. This difference was more marked in the animals receiving potassium chloride than in those receiving sodium chloride (Figure 9).

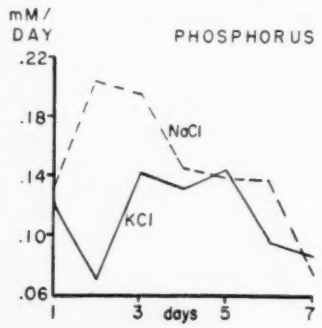

FIG. 7. AVERAGE PHOSPHORUS EXCRETION IN GROUPS K AND NA

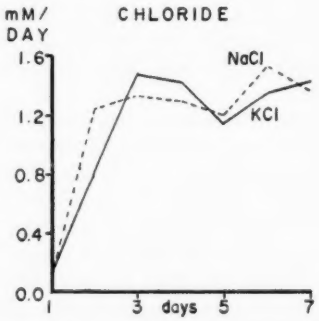

FIG. 8. AVERAGE CHLORIDE EXCRETION IN GROUPS K AND NA

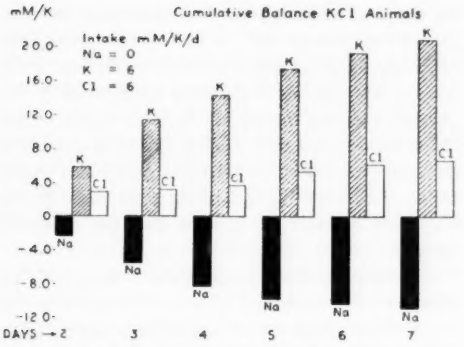

FIG. 9. CUMULATIVE BALANCE OF SODIUM, POTASSIUM, AND CHLORIDE IN POTASSIUM CHLORIDE TREATED ANIMALS

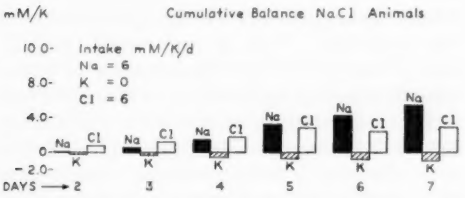

FIG. 10. CUMULATIVE BALANCE OF SODIUM, POTASSIUM, AND CHLORIDE IN SODIUM CHLORIDE TREATED ANIMALS

There were insignificant retentions of sodium and chloride and a very small loss of potassium in the animals that received sodium chloride (Figure 10).

Analysis of serum during and at the end of the experiment revealed complete correction of the extracellular hypochloremic alkalosis in those animals receiving potassium chloride (initial serum bicarbonate concentration = 30.0 mM/L, final serum bicarbonate concentration = 23.1 mM/L). By contrast those animals receiving sodium chloride showed the same bicarbonate concentrations at the end as at the beginning of the experiment (Table I).

TABLE II

Average muscle composition per 100 g. fat-free solids

Group	No.	H_2O	Cl	Na	K	Phos.	Nai
Normal (*10*)		340	7.1	10.0	48.9	32.4	2.4
Deficient	8	332	5.5	18.4	34.2	34.3	12.2
NaCl 36 mM/Kg.	8	332	4.81	18.1	33.0	35.5	13.0
KCl 6 mM/Kg.	7	330	5.8	17.5	36.7	35.2	11.2
KCl 12 mM/Kg.	6	327	5.7	13.7	40.9	35.0	7.8
KCl 18 mM/Kg.	7	320	5.7	12.1	43.6	34.5	6.3
KCl 27 mM/Kg.	4	322	5.97	10.7	42.0	35.9	4.3
KCl 36 mM/Kg	2	317	4.89	10.1	45.7		5.3

Analysis of muscle in both groups at the beginning of the experiment revealed the characteristic changes in composition noted previously in potas-

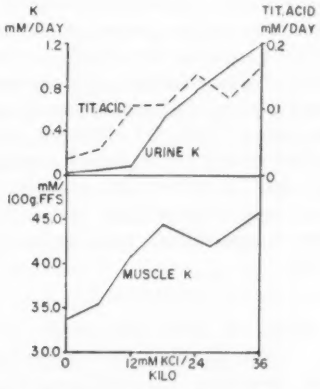

FIG. 11. RELATIONSHIP OF URINE POTASSIUM AND TITRATABLE ACIDITY TO CHANGE IN MUSCLE POTASSIUM DURING ADMINISTRATION OF POTASSIUM CHLORIDE

TABLE I

Average serum analyses

Group	No.	pH	H_2O	Na	K	Cl	HCO_3	[Na]	[K]	[Cl]	$[HCO_3]$
			g./L	*mM/L*	*mM/L*	*mM/L*	*mM/L*	*mM/Kg. of water in ultrafiltrate of serum**			
Normal (*10*)		7.28	935	143	5.4	103	21	145	5.4	115	23
Deficient	8	7.46	935	143	2.7	93	30	145	2.7	105	33
NaCl 36 mM/Kg.	8	7.46	935	143	2.9	96	30	145	2.9	108	34
KCl 6 mM/Kg.	7	7.50	935	141	3.3	96	26	144	3.3	109	29
KCl 12 mM/Kg.	6	7.39	935	141	4.3	103	23	144	4.3	116	26
KCl 18 mM/Kg.	7	7.39	935	141	4.7	104	23	144	4.7	117	26
KCl 27 mM/Kg.	4	7.43	935	141	4.4	101	24	143	4.4	114	27
KCl 36 mM/Kg.	2	7.45	935	141	4.6	105	23	143	4.6	118	25

* Donnan factor = 0.95.

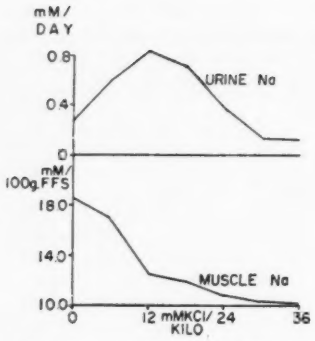

FIG. 12. RELATIONSHIP OF URINE SODIUM TO CHANGE IN MUSCLE SODIUM DURING ADMINISTRATION OF POTASSIUM CHLORIDE

sium deficiency (3). There was marked increase in muscle sodium and decrease in muscle potassium (Table II). The increase in intracellular sodium amounted to about two-thirds of the decrease in intracellular potassium as described earlier (3, 7). ($\Delta Na_i = -2/3\Delta K_i$). In group K, analysis of muscle during and at the end of the experiment revealed a gradual return toward normal composition (Table II). The fall in intracellular sodium during repair lagged somewhat behind the rise in intracellular potassium. The changes in muscle composition were reflected in equivalent changes in urinary excretion (Figures 11 and 12).

DISCUSSION

The results of these experiments present a paradox. Thus, although renal regulation must ultimately determine extracellular composition, the rats receiving potassium chloride recovered from metabolic alkalosis while the kidneys excreted a urine with greater titratable acidity and ammonia and lower bicarbonate content than the rats receiving sodium chloride. Furthermore, the rats receiving potassium chloride excreted chloride in excess of sodium plus potassium. If these excretions are considered from the point of view of extracellular fluid alone, recovery from alkalosis is not explained. The urine findings and the correction of extracellular alkalosis must then be the result of exchange of ions between extracellular fluid and muscle cell. In effect these potassium-deficient, alkalotic rats from the standpoint of the body as a whole had a deficit of fixed cation which was in excess of the deficit of fixed anion. Thus the chloride excretion during repair was greater than the sum of the sodium and potassium excretions. Likewise the deficit of potassium in the body was considerably greater than the excess of sodium.

The magnitude of these disturbances and the nature of the exchange of ions between cell and extracellular fluid are indicated by muscle analysis. Darrow and his associates (3), originally, and Muntwyler and Griffin (7), subsequently, demonstrated that, in potassium deficiency with alkalosis, the rise in intracellular sodium amounted to approximately two-thirds of the fall in intracellular potassium. Since normal muscle composition is restored by the administration of potassium chloride, approximately three potassium ions must have entered the cell for every two sodium ions leaving it during repair (Figure 13). If electrical neutrality is to be maintained, some cation other than sodium must have been exchanged for potassium. The data of Cotlove and his associates (8) indicate that the other cation exchanged is not calcium or magnesium. By exclusion the exchange of hydrogen ion from within the cell for potassium of extracellular fluid must have occurred. This exchange of hydrogen ion for fixed cation is identical with the concept of change in base-binding capacity of the cell anion, since the basis of alteration in base-binding capacity of anion is the substitution of hydrogen ion for fixed cation with alteration in dissociation of the compound that results.

$$H^+ + K^+ + A^- \rightleftarrows HA + K^+$$

The exchange of extracellular potassium for intracellular hydrogen lowers the extracellular bicarbonate concentration if potassium is administered along with a fixed anion such as chloride. By this exchange a strong acid is formed which reacts with extracellular bicarbonate to form carbon dioxide.

$$\underset{\text{(from cell in exchange for } K^+)}{H^+} + Cl^- + Na^+ + HCO_3^- \rightleftarrows Na^+ + Cl^- + \underset{\Uparrow}{\overset{CO_2 + H_2O}{H_2CO_3}}$$

It is likely that the interchange of hydrogen for potassium which is described is not a reaction which is suddenly initiated under these particular circumstances but rather is an established equilibrium, such as may occur in yeast (9). The net exchange described would result from an alteration in the equilibrium point with an altered concentration of reactants on each side of the membrane.

During repair of alkalosis with potassium chloride the magnitude of the change in extracellular composition which is imposed by the transfer of electrolyte from the cell can be readily calculated from the changes in intracellular composition. The net exchange of sodium, potassium, chloride and hydrogen ions at the membrane will be reflected in changes in extracellular composition and in altered excretion of anions and cations by the kidney.

In the calculation of the theoretical balance of electrolyte from changes in muscle composition three quite probable assumptions are made. First, muscle is assumed to make up 50 per cent of the body weight. Second, extracellular fluid is assumed to be 20 per cent of the body weight after correction with potassium chloride. Third, the fat-free solids (FFS) of muscle are considered to remain constant over a short period of time. The actual body balance of chloride, sodium and potassium after 18 mM per kilogram of potassium chloride had been injected (three days) is of the same order of magnitude as the theoretical balance calculated from observed changes in muscle composition during the same period of study. The balances are compared for this period of study rather than at the end of one week to minimize cumulative errors arising from undetermined losses and to emphasize the large changes in body composition which occur rapidly. It is assumed that the determined balance of chloride, 3.7 mM/Kg., remains in extracellular fluid. This balance of extracellular chloride is used to calculate the change in extracellular volume according to the method of Darrow (10).

Calculations:[6] *per kilogram of body weight*

$(Cl)_2 = (H_2O)_{e_2} \times [Cl]_2$

$= .200 \times 117 = 23.4$ mM

$(Cl)_1 = (Cl)_2 - \Delta\,(Cl)$

$= 23.4 - 3.7 = 19.7$ mM

$(H_2O)_{e1} = \dfrac{(Cl)_1}{[Cl]_1}$

$= \dfrac{19.7}{105.} = 0.188$ Kg.

$(Na)_2 = (H_2O)_{e_2} \times [Na]_2 + FFS \times (Na)_{i_2}/100$ g. FFS

$= .200 \times 144 + \dfrac{120}{100} \times 6.3$

$= 36.4$ mM

$(Na)_1 = (H_2O_e)_1 \times [Na]_1 + FFS \times (Na)_{i_1}/100$ g. FFS

$= .188 \times 145 + \dfrac{120}{100} \times 12.2$

$= 41.9$ mM

$\Delta\,(Na) = (Na)_2 - (Na)_1 = -5.5$ mM

Likewise

$\Delta\,(K) = (K)_2 - (K)_1 = +11.6$ mM

The theoretical balance of chloride is by assumption equal to the actual balance = + 3.7 mM/Kg.

Theoretical Na Balance = − 5.5 mM/Kg. Actual Na Balance = − 8.2 mM/Kg.

Theoretical K Balance = + 11.6 mM/Kg. Actual K Balance = + 14.3 mM/Kg.

If the difference between the decrease in sodium within muscle ($\Delta(Na)_i$) and the increase in potassium ($\Delta(K)_i$) during repair with potassium chloride is assumed to be mainly hydrogen ion transferred from cells to extracellular fluid, it is possible to calculate the theoretical decrease in the amount of extracellular bicarbonate (theoretical $\Delta(HCO_3)_e$) which results from extrarenal correction. The nomogram of Singer and Hastings (11) is also used to correct for the buffering capacity of whole blood with the observed change in pH.

Calculation: per kilogram of body weight

$(HCO_3)_{e_2}$	$= (H_2O)_{e_2}$	$\times [HCO_3]_{e_2}$		
	$= .200$	$\times 26$	$=$	5.2 mM
$(HCO_3)_{e_1}$	$= (H_2O)_{e_1}$	$\times [HCO_3]_{e_1}$		
	$= .188$	$\times 33$	$=$	6.2 mM
$\Delta\,(HCO_3)_e$	$= (HCO_3)_{e_2}$	$- (HCO_3)_{e_1}$	$=$	− 1.0 mM
Δ Buffer base (Calculated)			$=$	− 0.3 mM
Actual $\Delta\,(HCO_3)_e$			$=$	− 1.3 mM/Kg.

Calculation of theoretical $\Delta\,(HCO_3)_e$ *from H transport*

$(Na)_{i_2} = FFS \times (Na)_{i_2}/100$ g. FFS

$= \dfrac{120}{100} \times 6.3 = 7.6$ mM

[6] ( ) refers to the total amount in mM or Kg. [ ] refers to concentration in ultrafiltrate of serum in mM per kilogram of water. (H_2O) refers to volume of fluid in kilograms. FFS refers to fat-free solids of muscle in grams and equaled 120 g./Kg. of rat. Subscripts "e" and "i" refer to extracellular and intracellular, respectively. Subscripts "1" and "2" refer to values before and after correction, respectively.

$$(Na)_{i_1} = FFS \times (Na)_{i_1}/100 \text{ g. FFS}$$
$$= \frac{120}{100} \times 12.2 = 14.6 \text{ mM}$$
$$\Delta (Na)_i = (Na)_{i_2} - (Na)_{i_1} = -7.0 \text{ mM}$$

Likewise

$$\Delta (K)_i = (K)_{i_2} - (K)_{i_1} = 11.2 \text{ mM}$$
$$\text{Theoretical } \Delta (HCO_3)_e = -\Delta (Na)_i - \Delta (K)_i$$
$$= -4.2 \text{ mM/Kg.}$$
$$\text{Theoretical } \Delta (HCO_3)_e = -4.2 \text{ mM/Kg. Actual}$$
$$\Delta (HCO_3)_e = -1.3 \text{ mM/Kg.}$$

As can be seen from the above calculations, the fall in extracellular bicarbonate calculated from change in muscle composition is considerably greater than that actually measured and is equivalent to most of the bicarbonate normally present in extracellular fluid. The reason for the discrepancy is obvious in view of the urinary findings during repair with potassium chloride. The presence of low urine pH, high titratable acid and ammonia excretion, as well as low bicarbonate output, indicates some renal compensation to prevent extracellular acidosis which results from transfer of large quantities of hydrogen from the cells. The hydrogen ion excreted as titratable acid and the difference in ammonia excretion between the two groups of animals may be considered equivalent to potential extracellular bicarbonate (a probably valid assumption since it represents fixed cation reabsorbed without fixed anion). The actual change in extracellular bicarbonate plus the bicarbonate conserved by the kidney (the sum = Effective $\Delta(HCO_3)_e$) is of the same order of magnitude as the potential theoretical fall in extracellular bicarbonate as derived from muscle analysis.

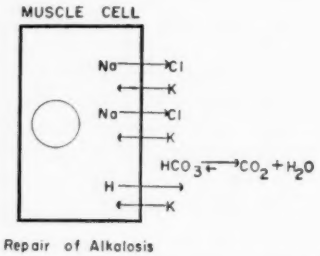

FIG. 13. CELL TRANSFER CORRECTING ALKALOSIS WITH ADMINISTRATION OF POTASSIUM CHLORIDE

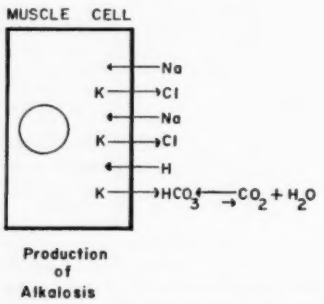

FIG. 14. CELL TRANSFER PRODUCING ALKALOSIS IN POTASSIUM LOSS

Actual	$\Delta (HCO_3)_e = -1.3$
+	Tit. Ac. $= -1.0$
+	$NH_3 = -2.5$
Effective	$\Delta (HCO_3)_e = -4.8$ mM/Kg.
Theoretical	$\Delta (HCO_3)_e = -4.2$ mM/Kg.

The data presented illustrate the correction of extracellular alkalosis associated with potassium deficiency by the transfer of electrolyte between muscle cells and the surrounding fluids. The converse reactions between muscle cells and extracellular fluid must occur during the development of this type of change in body composition, *i.e.*, in alkalosis associated with potassium deficiency. Figure 14 is a schematic representation of the transfers of electrolyte during development of potassium deficiency. It is, of course, obvious that dissolved carbon dioxide (carbonic acid) produced by the cells does not represent a load to be excreted by the kidney and that with intact respiratory mechanisms carbon dioxide is of little importance in altering the electrolyte pattern of extracellular fluids. The exchange of fixed cation from the cell for hydrogen ion, which is derived from the dissociation of carbonic acid of extracellular fluid, produces the same effect on extracellular composition as would the addition of potassium bicarbonate to the extracellular space. Under these conditions extracellular bicarbonate concentration must rise unless the kidneys excrete fixed cation in excess of fixed anion.

It is clear from the calculations of the theoretical bicarbonate reduction which is produced by exchange at the cell membrane during recovery from potassium deficiency that a corresponding production of bicarbonate occurs during genesis of potassium deficiency. It is not clear, however, why the kidneys in potassium deficiency do not compensate for this load of fixed cation in excess

of fixed anion and thereby preserve extracellular electrolyte concentration. A satisfactory explanation of this failure in renal regulation of extracellular composition would probably also explain the failure of sodium chloride to correct the extracellular alkalosis associated with potassium deficiency.

If the changes in composition that have been described for muscle in alkalosis associated with potassium deficiency also apply to the cells of the distal tubule of the kidney, these abnormalities in renal adjustment of alkalosis could be explained. These cells would then contain an excess of sodium and hydrogen ions and a deficiency of potassium. Excretion of sodium in excess of chloride would thus be impaired as postulated by Berliner, Kennedy, and Orloff (5). Analysis of rats with potassium deficiency did not reveal such a change in the composition of whole kidneys (12). If such a change occurs in certain cells, it is obscured by the method of analysis which, of necessity, included all cells, tubular urine, and extracellular fluid.

SUMMARY

Alkalosis associated with potassium deficiency was produced in rats by injection of desoxycorticosterone acetate while on a diet deficient in potassium and containing an excess of sodium over chloride. Over a period of six days one group of these animals received injections of 6 mM/Kg. of potassium chloride daily, while another group received 6 mM/Kg. of sodium chloride per day.

The group receiving potassium chloride restored serum and muscle composition to normal over a period of three to six days. This repair was accompanied by an increase in urinary titratable acidity and ammonia. The sodium chloride group showed no correction of serum or muscle composition and there was no change in urinary titratable acidity and ammonia over this period.

It is pointed out that the repair of alkalosis in the rats receiving potassium chloride is accounted for by the transport of electrolyte between muscle and extracellular fluids. Since the gain of potassium by the cells is greater than the loss of sodium from the cells, hydrogen ion must be exchanged to preserve electrical neutrality. The calculated net exchange of hydrogen ion from cell to extracellular fluid is sufficient to account for the reduction in serum bicarbonate concentration and the increase in urinary titratable acidity and ammonia.

A general theory of extrarenal modification of acid-base balance is formulated from these findings.

REFERENCES

1. Gamble, J. L., Chemical Anatomy, Physiology and Pathology of Extracellular Fluid. Harvard University Press, Cambridge, Mass., 1947.
2. Pitts, R. F., and Lotspeich, W. D., Bicarbonate and the renal regulation of acid base balance. Am. J. Physiol., 1946, **147**, 138.
3. Darrow, D. C., Schwartz, R., Iannucci, J. F., and Coville, F., Relation of serum bicarbonate concentration to muscle composition. J. Clin. Invest., 1948, **27**, 198.
4. Darrow, D. C., Renal function. Transactions of the Third Conference of The Josiah Macy, Jr. Foundation, 1951, in press.
5. Berliner, R. W., Kennedy, T. J., Jr., and Orloff, J., Relationship between acidification of the urine and potassium metabolism; effect of carbonic anhydrase inhibition on potassium excretion. Am. J. Med., 1951, **11**, 274.
6. Conway, E. J., Microdiffusion Analysis and Volumetric Error. Lockwood & Son, Ltd., London, 1950, 3rd ed.
7. Muntwyler, E., and Griffin, G. E., Effect of potassium on electrolytes of rat plasma and muscle. J. Biol. Chem., 1951, **193**, 563.
8. Cotlove, E., Holliday, M. A., Schwartz, R., and Wallace, W. M., Effects of electrolyte depletion and acid-base disturbance on muscle cations. Am. J. Physiol., 1951, **167**, 665.
9. Conway, E. J., Biological interlinkage of acid-alkali formation with ion transport and synthetic reduction. Irish J. M. Sc., 1949, No. 288, 787.
10. Darrow, D. C., Retention of electrolyte during recovery from severe dehydration due to diarrhea. J. Pediat., 1946, **28**, 515.
11. Singer, R. B., and Hastings, A. B., An improved clinical method for the estimation of disturbances of the acid-base balance of human blood. Medicine, 1948, **27**, 223.
12. Darrow, D. C., and Cooke, R. E., Kidney composition in potassium deficiency. To be published.

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